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Reproductive Biology and Seed Production of the Tropical Abalone *Haliotis varia* Linnaeus (Gastropoda)

THESIS SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Doctor of Philosophy

IN MARICULTURE

OF THE
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VERSOVA, MUMBAI – 400 061

By

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SEPTEMBER 2000

Dedicated to

My

Late Father & Baby

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)

भाकृ अनुप
ICAR

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CERTIFICATE

This is to certify that the thesis entitled **"Reproductive biology and seed production of the tropical abalone *Haliotis varia* Linnaeus (Gastropoda)"** is an authentic record of the research work carried out by **Mr. T.M. NAJMUDEEN** under my scientific supervision and guidance at Central Marine Fisheries Research Institute, Kochi, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** of the Central Institute of Fisheries Education (Deemed University), Mumbai, and no part thereof has been presented for the award of any other degree, diploma or associateship in any University.

A.C.Victor

Kochi – 682 014.
September, 2000.

DR A.C.C. VICTOR
(Supervising Guide)

DECLARATION

I, **T.M. NAJMUDEEN**, do hereby declare that this thesis entitled **Reproductive biology and seed production of the tropical abalone *Haliotis varia* Linnaeus (Gastropoda)** is a genuine record of the research work done by me under the scientific supervision of **Dr A.C.C. VICTOR,** Senior Scientist, TRC of CMFRI, Tuticorin, and has not previously formed the basis for the award of any degree, diploma or associateship in any university.

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Chapter I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The vast expanse of ocean has often been seen as a limitless source of animal protein for mankind. As this source is depleting in recent years due to more intense fishing pressure, maritime countries have given priority to aquaculture development with the objective of preserving and increasing their natural resources. The expansion of culture industry of any species chiefly relies on an improved understanding of the biology of the species, particularly on spawning characters. Dependence on the natural seed resources for culture is risky because it is extremely variable in quality and quantity and off-season availability. So artificial seed production is the only alternative for the expansion of aquaculture especially when the distribution of the species is limited. Diligent work by fishery scientists in many parts of the world is bringing the reproductive process and seed production of more organisms under control.

Molluscs are one of the most compact groups of animals with more species known from marine environments than of any other animal phylum. In number of species, the mollusca are the second phylum to the Arthropoda, comprising about 80000 species. A major part of the world marine aquaculture production is made up of molluscs including clams, cockles, oysters, mussels, scallops and abalones. In aquaculture production, molluscs are the third largest commodity in the Asia-Pacific region providing about 16% of the total. Three quarters of molluscs are gastropods with about 1650 genera. Gastropods are among the most conspicuous sea animals, and species of limpets, snails and slugs are found in all the marine habitats. Members

of this class have one shell, as opposed to clams and oysters with two. The most valuable gastropod from an epicurean point of view is certainly the abalone.

Abalones, commonly known as ear shell, are economically important marine gastropods belonging to the genus *Haliotis*. There are about 100 species of abalones in the world. They are found in both the hemispheres, but the larger varieties exist in the temperate regions, while the smaller ones live in tropical and arctic regions. Because these animals have been of commercial value since ancient times much has been written about their natural history beginning with Aristotle (Crofts, 1929). The first fisheries of abalone were in China and Japan over 1500 years ago, but it is only in the last 30 years that the fisheries for abalone have burgeoned worldwide and become economically important in many countries.

Abalone meat is highly priced and contains about 20% protein. They have blue-gray mother-of-pearls that can be made in to several types of ornaments. It is considered to be one of the best and most valuable seafoods in various parts of Asia. Abalone is valuable as an ingredient in Chinese medicine (Guo *et al.*, 1999). The viscera can be made into good quality glue. Abalones can also be used to produce pearls. The first recorded reference to abalone pearls occurs in one of Japan's oldest historical writings (Shirai, 1970). The nacre of abalone shell is often multihued in tones of silver, orange, pink, green, blue and lavender. The colour play tends to be very beautiful in these pearls since the nacre that is produced by abalone is thick and can reflect the full spectrum of rainbow colours. The quality of abalone pearls as reflected in their surface texture, is superior to the pearls produced in fresh water mussels and comparable to the best pearls cultured in marine pearl oysters (*Pinctada* sp.).

Cultivation of abalone has spanned nearly a 50-year period. Pioneering efforts to cultivate North American Haliotids began in 1940 (Carlisle, 1962). The 1970s witnessed a continued interest in red abalone cultivation in California. In 1972, there were only two nations studying abalone aquaculture. Improvement of hatchery and culture technology, and expansion of culture area has continued since this time. Consequently abalone aquaculture has rapidly developed and production has greatly increased, particularly in Taiwan and Japan. Japan is at present the acknowledged leader in developing techniques for the mass production of juveniles.

Although large abalones are very popular in many Asian countries, small or cock tail size abalone of 40-70 mm shell length are also popular in some countries. Chen (1989) states that in Taiwan, small abalones are preferred to large species owing to their delicate flavour, appropriate size for banquets and price.

The total world production of abalones through aquaculture has increased in 1997 to 2484 tonnes from 2179 tonnes in 1996 (FAO, 1999). China and Taiwan contributed to around 89% of the total abalone production. USA contributed 265 mt to the world abalone aquaculture production in 1997. *Haliotis discus hannai* is the primary abalone species supporting abalone culture industry around the globe accounting for more than 70% of the world abalone aquaculture production.

The pearl culture in abalone appears to be a fairly recent enterprise. Unlike oysters, cultivating pearls in abalone is extremely difficult. The pearl nucleus is attached to the inside of the shell in a way in which the abalone will coat the nucleus with nacre, the pearl secretion, without being able to expel it. There is a great chance for the rejection of the nucleus by the some times violent movements of abalone's large foot.

As the culture of round pearls in abalone met with only partial success due to the nucleus dislodgement and infections precipitated by the muscular foot, most farmers are now attempting to culture both 'mabe' and 'blister' pearls in abalone. A mabe pearl is a dome shaped pearl cut from the shell around the pearl's perimeter. A blister pearl is cut from the shell, leaving some of the surrounding shell to accent the pearl. With abalone pearls, this mother-of-pearl border can add greater variety of colours and create a very interesting gem.

The red abalone, *Haliotis rufescens* is the largest of the abalones in the world, often reaching lengths greater than 27.5 cm and weighing over 1.7 Kg. Red abalone has traditionally been the most popular and commercially important species in California. The other important species in North America include *H. fulgens* (green abalone), *H. corrugata* (yellow abalone), *H. sorensini* (white abalone), *H. cracherodii* (black abalone) and *H. kamtschatkana* (pinto abalone). Ezo awabi, *H. discus hannai*, found in Japan, is probably the most thoroughly studied abalone species in the world growing to between 18 and 20 cm in shell length. The other species found in Japan are *H. discus* (kuro awabi), *H. diversicolor supertexta* (tokobushi) and *H. gigantea* (madaka). *H. ruber* is the major Australian abalone species with *H. laevigata* and *H. roei*. *H. tuberculata* or ormer is the only commercial species in Europe. *H. midae* represents abalones in South Africa.

Unlike the temperate species mentioned above, the tropical abalone species are smaller in size and less in abundance. The major tropical abalones are *H. diversicolor supertexta*, *H. asinina*, *H. ovina* and *H. varia*. Of these, *H. asinina* enjoys distribution in Japan, Thailand and Philippines. The other two species are

abundantly distributed along the Andaman Sea coast of Thailand. All the tropical abalone species grow less than 10 cm in shell length.

While abalones occur in many parts of the world, the species large enough to support commercial fishery are found along temperate coasts. The natural history of abalone facilitates over fishing, and this has been a problem in every abalone producing country. In Indian Ocean, abalones are found from the Arabian Sea and the Persian Gulf to the Bay of Bengal around Andaman and Nicobar Islands as well as near Sri Lanka. In India, abalone is represented by only one species, *Haliotis varia*. It grows to a maximum shell length of 80 mm. It is moderately distributed in Gulf of Mannar along Pamban and Tuticorin areas of Southern Tamil Nadu. Hornell (1917) states that abalones (*Haliotis*), which are highly valued in other parts of the world and occurring there in great abundance are scarce and of small size in India, making it unnoticeable by the fishermen and researchers. But owing to the importance of abalones in world aquaculture scenario, it is important to initiate research on the abalones in India.

Aquatic animals exhibit a wide variety of reproductive strategies; however, virtually all the important aquaculture species rely on some form of sexual reproduction. Successful aquaculture is often dependant on the ease with which culture animals can be reproduced in captivity. Like wise, the knowledge on the breeding cycles of wild abalones is vital to the management of culture operations. Unless captive spawning and rearing are achieved, the culturist has little or no control on the genetic make up of the stock and is thus unable to attempt to improve the characteristics of the animals through selective breeding. Seed production is the major turning point in the domestication of animals assuring the opportunity to

culturists to produce animals at a higher rate than that can be produced in the wild. Success in captive spawning and larval rearing has been achieved for the majority of the abalone species under culture today.

The termination of the planktonic stage of larvae is the most critical event in the life history of abalones. The transformation from abalone larva to juvenile involves two distinct processes: Settlement and metamorphosis (Crisp, 1974). Settlement has been described as the behavioural change typically characterized by the active searching for certain environmental factors such as specifics, prey species, algae and inorganic cations (Hadfield, 1984). It is a reversible event that may occur several times prior to metamorphosis. Metamorphosis is a non-reversible phenomenon that involves dramatic anatomic and physiological changes in larvae, ultimately yielding juvenile individuals. Larvae of the red abalone, *Haliotis rufescens* has been shown to settle and metamorphose in response to a number of biological substrates (Morse, *et al.*, 1979a, 1980a,b; Seki and Kan-no, 1981a,b; Slattery, 1992). Larvae of many species of *Haliotis* attach to the substratum and metamorphose in the presence of benthic diatoms and conspecific mucus (Seki and Kan-no, 1981a: Slattery, 1992).

The purpose of the present study is to investigate the mechanism of gametogenesis and to determine the annual reproductive cycle of *Haliotis varia* from Gulf of Mannar, the South East Coast of India. An effort is done to demonstrate that the seed production of *H. varia* can be achieved in controlled conditions. The thesis is presented in five chapters. The first chapter is an introduction, highlighting the importance of abalones and the present work. The taxonomic position of *Haliotis varia* was also mentioned along with its habitat description and distribution.

In the second chapter the reproductive biology of *H. varia* is described with the help of light and electronmicroscopic studies. Various procedures such as calculation of sex ratio, gonadosomatic index, fecundity, size at first sexual maturity, hepatosomatic index and the construction of oocyte size frequency profiles are employed to study the reproductive biology and annual reproductive cycle of *H. varia* at two stations; Tuticorin and Mandapam in Gulf of Mannar. The information on variations in the hydrographic parameters like salinity, temperature, pH and dissolved oxygen in the two stations is also presented.

The process of gonad maturation is often associated with the accumulation of nutrients in the storage organs and its subsequent mobilization to the gonad. The knowledge on the variations in different biochemical components in different tissues of the animal during that period will be helpful in determining the annual reproductive cycle. Hence an attempt is made to reveal the pattern of movement of biochemical parameters during the maturation process of *H. varia* using various biochemical procedures and it is presented in Chapter III.

In the fourth chapter, the seed production of *H. varia*, which was achieved in controlled conditions in the laboratory, is documented with the help of photographs of different larval stages. The larval rearing and the successful inducement of settlement and metamorphosis of larvae of *H. varia* are described in detail.

The thesis is concluded by giving a summary of the work done in chapter V followed by the list of references cited in the text.

THE SYSTEMATIC POSITION OF THE ABALONE STUDIED

Phylum : Mollusca
Class : Gastropoda
Subclass : Prosobranchia
Order : Archaeogastropoda
Superfamily : Pleurotomariacea
Family : Haliotidae
Genus : *Haliotis*
Species : *varia*

Generic characters: Shell ear shaped, depressed and loosely coiled. Spire eccentric and protruding only a little or not at all. A spiral row of holes on the left side of body whorl, sometimes on tubular projections, the last few remaining open. Aperture broad, occupying most of the under side with a remarkably thickened inner lip. Interior nacreous, with a big sub central muscle scar. No operculum. Head with a short snout and large rounded tentacles bearing eyes on short lateral stalks of their outer bases. Foot broad and ovate, very strong. A sensory ridge along the edge of the foot, bearing a series of tentacles. Two gills, the right one slightly reduced in size.

Food: Abalones eat marine algae. The adults feed on loose pieces of algae drifting with the surge of the current. Large brown algae such as giant kelp, bull kelp and elk kelp are preferred in temperate regions, although most others may be eaten at various times. Abalone tends to stay at one location waiting for food to drift by. However, they will move daily, seasonally or when food become scarce for a longer period. The colour binding on many abalone shells is due to the changes in the types of algae eaten. Juvenile abalone graze on rock encrusting coralline algae and on diatoms and bacterial films. As they grow, they increasingly rely on drift algae.

Haliotis varia Linnaeus 1758

Synonyms: *H. coccinea* Reeve, 1846; *H. semistriata* Reeve, 1846; *Sanhaliotis varia* (Linnaeus, 1758).

Common names: Variable abalone, sea ear, tropical abalone.

Vernacular names: Tamil- *Kadal kaathu, Thulkachi kaathu.* Malayalam- *Kadal chevi.*

Species characters: Shell thick, elongate-ovate in out line, rather inflated, not keeled in periphery of left side. Outer sculpture extremely variable, comprising irregular radial folds crossed by low, rounded spiral ribs of different thickness, some of them weakly knobbed. Periphery of left side with a few nodulose spiral cords. Holes rounded to oval, on slightly elevated tubercles, the last four or five holes open. Ridge of inner lip well developed, its posterior part hiding the internal coils of spire. Outer lip regularly convex. Inner side of shell is somewhat reflecting the variable outer sculpture, muscle scars sometimes well developed (FAO, 1998).

Habitat: Among rocks and under stones, in rocky shores and coral reef areas. Littoral to shallow subtidal depths.

Distribution: Wide spread in the Indo West Pacific, from East Africa, including the Red Sea to Malaysia; north to Japan and south to southern Queensland; and along the Andaman Sea coast of Thailand. In India distribution restricted to Gulf of Mannar, on the South East coast of Tamil Nadu.

Description of the study areas: The collection sites Tuticorin and Mandapam belong to the southern Tamil Nadu and is situated along the coast of Gulf of Mannar, separated by a distance of about 140 kilometers (Plate II and III1) Tuticorin is situated at a latitude of 08^0 45' N and longitude of 78^0 12' E. Here the abalones are attached to the granite stones along the coral bed inside the harbor basin (Plate VI1). The wave

action in this area is moderate. The Mandapam collection site is situated at longitude 09^0 16' N and longitude 79^0 12' E. The abalones are distributed along the 'paars' behind the Krusadi, Pullivasal, Poomarachan and Manauli islands (Plate VI2). Unlike Tuticorin station, here the wave action is strong. Abalones are found to attach to the dead corals and in the crevices of coral stones.

Chapter II

REPRODUCTIVE BIOLOGY

1. Introduction
2. Materials and Methods
3. Results
4. Discussion

INTRODUCTION

Detailed information on the reproductive biology of the cultured species is vital to the culturist enabling him to rear them under most favourable conditions to achieve maximum success in induced maturation and spawning. This information can be used to design efficient and effective techniques for conditioning, spawning inducement, larval development and settlement. Studies on reproduction are also useful in formulating fishery management regulations for commercial species. Knowledge of breeding seasons is essential for the estimation of growth rates, particularly of young stages. The relationship between age, fecundity, and the minimum size at maturity must be taken into account when minimum fishable size is determined.

Reproductive pattern in archaeogastropods is of broadcast spawning in which mature sperms and eggs are released into the surrounding media without any secondary modification. Among Prosobranch molluscs, the simplest reproductive system is encountered in archaeogastropods (Giese and Pearse, 1977). The reproductive system is similar in both sexes and consists of a single gonad with a gonoduct that leads to the right kidney. In most prosobranchs, gametogenic activity throughout the reproductive cycle is synchronous in a population and can be divided into distinct maturation stages. In some species, however, there are population differences in gametogenesis between sexes, and in others the population has no synchrony.

The reproductive cycle is defined as the time interval between successive spawnings in a population. The methods to study the reproductive biology range from

histology to visual staging based on the external appearance of the gonad. Between these two are measurements of oocyte size, staging based on the appearance of whole oocytes and gonad indices. Except the oocyte size measurements, all other methods are applicable to males also.

Histological studies are the most accurate technique, which provide very precise information on oocyte and spermatocyte developmental stages. But it is time consuming and expensive. Staging based on the external appearance of the gonad is the simplest and most rapid method, but it may be subjective and its accuracy is uncertain. Staging based on the appearance of the whole oocytes and spermatocytes can be a useful alternative, but may be inaccurate with oocytes in transitional stages of development. Oocyte size is used on its own to measure development but gives little information on the physiological status of the ovaries. Gonad indices (gonad size relative to body size) provide a useful insight into changes in gonad size. However, gonad indices may be biased when samples of animal of different body sizes are compared. Gonad index is a convenient representation of the reproductive state of the abalone (Webber and Giese, 1969).

Mere examination of the morphological characters of male and female gonad will not give actual maturity condition of the gonad. The reproductive cycle of a species can be determined by monitoring changes in gonad histology (Ault, 1985). Examination of histological sections of gonads provides more information on reproduction than is possible from gonad indices alone. Investigating the mechanism of spermatogenesis and oogenesis and their relation to the morphological characters of the different stages of gonad is important for the selection of brood stock for culture. Subjectively assigning a gonad development stage is the simplest analysis of

histological sections. The number of stages assigned to gametogenesis varies depending on researcher and sex of the abalone. Tomita (1968) classified spermatogenesis in abalone into three stages: spermatogonium, spermatocyte and spermatid; and oogenesis into seven stages: 1) Oogonium (3-5 μm in diameter), 2) Chromatin-nucleolus (10 μm), 3) Yolkless (50 μm), 4) Oil drop (50-90 μm), 5) Primary yolk globule (150 μm), 6) Secondary yolk globule (150- 180 μm) and 7) Mature (150-200 μm).

The number of gonad maturation stages assigned can range from 5 to 10 depending on how rapidly and completely the animal spawns (Grant and Tyler, 1983). Giese (1959) and Boolootian (1966) called them 1) activation (initiation of gametogenesis), 2) gametogenesis, 3) increase in gonad size due to increase in the number or size of gametes, 4) spawning, 5) resorption of unspawned gametes and 6) resting. Tomita (1968) called them premature, mature, spawning, spent and recovery; Lee (1974) called them multiplication, growing, mature, spent and recovery. Ault (1985) called the gonad maturation stages preproliferative, proliferative, new stalk, old stalk and free; and Giorgi and DeMartini (1977) called them active, ripe, partially spawned, spent and necrotic.

Direct evidence on gametogenic states is obtained from microscopic examination of gonad tissue. Because of the ease of the measurement, however, relative gonad size (gonad index) is more often used as a measure of gonad maturity. It is the simplest and most frequently used method to represent the reproductive cycle. This index can be estimated on the basis of dry weight, wet weight or caloric content. In prosobranchs the gonad index appears to be a valid measure of gametogenic state (Giese and Pearse, 1977). A ripe gonad may make up 15 to 20% of the body weight

in a fully mature abalone and remains this size until spawning rapidly decreases the gonad size (Webber and Giese, 1969).

For any study on the reproductive cycle, the determination of stage of first sexual maturity in different populations is a necessary prerequisite. The minimum age of maturity in different abalone species have been studied (Newman, 1967; Poore, 1973; Hayashi, 1980; Bussarawit *et al.*, 1990; Wood and Buxton, 1996). Difference in the attainment of sexual maturity among different populations has been recorded in the wedge clam *Donax cuneatus* (Nayar, 1955 and Talikhedkar *et al.*, 1976).

Fretter and Graham (1964) noted that in dioecious molluscs, females tend to be more numerous than males and this is especially evident in older populations. The sex ratios of many haliotids have been studied and in majority of the cases observed, the sexes were found to be almost equally distributed (Stephenson, 1924; Crofts, 1937; Sinclair, 1963; Newman, 1967; Poore, 1973; Bussarawit *et al.*, 1990).

The fecundity of the abalone is a life history trait that is important for modeling exploited stocks (Sluczanowski, 1984), and for artificial spawning and mariculture. Most of the temperate abalone species are larger in size than the tropical abalone species, and hence the fecundity has been found to be very high (Newman, 1967; Poore, 1973; Hayashi, 1980; Shepherd *et al.*, 1992). In the tropical species, *H. varia*, which is smaller than the temperate species, the fecundity reported, was lesser than that of the temperate species (Bussarawit *et al.*, 1990). The fecundity of the Spanish abalone, *H. coccinea canariensis* that grows to a maximum size of 8 cm in shell length, was found to range between 11000 and 70000 (Pena, 1975).

Reproductive biology of Indian molluscs has been studied extensively during the last three decades. No study on the reproductive biology of abalone has been

reported from India to date, whereas in the other countries, where *Haliotis* species have been fished commercially for many years, research on their reproductive biology is extensive. Most workers have concentrated on the determination of the spawning seasons, such as of *H. discus* in Japan (Ino and Harada, 1961), *H. cracherodii* in California (Boolootian *et al.*, 1962) and of H. *iris* in New Zealand (Poore, 1973). More ample studies done in South Africa (*H. midae*) include estimations of fecundity (Newman, 1967).

The other *Haliotis* species studied for their reproductive cycle and gametogenesis during the early periods were *H. diversicolor supertexta* (Oba, 1964), *H. discus* (Tomita, 1967 & 1968), *H. cracherodii* (Webber and Giese, 1969), *H. discus hannai* (Yahata and Takano, 1970), *H. rufescens* (Young and DeMartini, 1970 and Giorgi and DeMartini, 1977), five Australian abalone species (Shepherd and Laws, 1974), *H. aquatilis* (Okuno *et al.*, 1978), *H. diversicolor diversicolor* (Takashima *et al.*, 1978), *H. pustulata* (Pearse, 1978), *H. tuberculata* (Hayashi, 1980), *H. kamtschatkana* and *H. walallensis* (Hahn, 1981) and *H. roei* (Wells and Keising, 1989).

Sufficient literature is available in the last two decades on the spawning and gametogenic cycle of different abalone species worldwide. Spawning pattern in the British Columbia population of *H. kamtschtkana* is given by Breen and Adkina (1980). Brown (1981) studied the sexual maturity of the Californian red abalone *H. rufescence* reared in Chile. Martinez (1983) estimated the fecundity in *H. rufescence* from Rosario Bay, Baja California. Study on the reproductive cycle of disk abalone, *H. discus hannai* was carried out by Liu *et al.* (1985). Quintanilla *et al.* (1985) have investigated annual reproductive cycle of three abalone species in Baja California using the variation in the gonad index.

Bang and Hahn (1993) have analyzed the influence of water temperature on the spawning and development of the abalone, *Sulculus diversicolor aquatilis*. Hahn (1994) studied the gametogenic cycle of *H. discus hannai* during conditioning with effective accumulative temperature. Reproduction and spawning pattern of Paua, *H. iris* have been studied in different parts of New Zealand (Hooker and Creese, 1995; Wilson and Shiel, 1995; McShane and Neylor, 1996). Studies on the reproductive biology of *H. mida*e on the east coast of South Africa were carried out by Wood and Buxton (1996).

The other gastropods studied for their reproductive biology and annual reproductive cycle in recent years include *Cellana grata* and *Ptelloidea pygmaea* (Liu, 1994), *Buccinum cyaneum* (Miloslavich and Dufresne, 1994), Umbonium *costatum* (Noda *et al.*, 1995), *Strombus pugilis* (Reed, 1995), *Planaxis sulcatus* (Oghaki, 1997), *Fusinus eloster* (Miloslavich and Penchaszadeh, 1997), *Dendropoma petraeum* (Calvo *et al.*, 1998), *Cypraea caputdraconis* (Osorio *et al.*, 1999) and *Siphonaria pectinata* (Ocana and Emson, 1999).

In Indian waters, investigation on reproductive cycle has been made on a number of bivalve molluscs (Rao, 1951, 1956; Durve, 1965; Nagabhushanam and Thalikhedkar, 1977; Rajapandian and Rajan, 1983; Joseph and Madyastha, 1982 and 1984; Mane and Nagabhushanam, 1976 and 1988; Narasimham 1988; Sukumar and Joseph, 1988; Victor and Subramoniam, 1988). Some of the works reported in India recent years include gastropods as well as bivalve molluscs. Breeding biology of Babylon snail *Babylonia spirata* has been studied by Kannapiran and Edward (1996). Reproductive cycle of the salt marsh snail *Pythia plicata* was studied by Shanmugam (1996). Shanmugam (1998) also studied the reproductive cycle of the ellobiid snail

Cassidula nucleus. Palaniswamy (1993) studied the seasonal gonadal changes and spawning of the oyster *Crassostrea ma*drasensis from Tuticorin. Maturation and sex ratio in the edible oyster *Crassostrea madrasensis* from Bay of Bengal were studied by Alam and Das (1998).

Although, several aspects of the biology of Indian molluscs have been investigated in recent years, comparatively little is known of their reproductive biology other than basic knowledge of breading seasons and annual reproductive cycle. No previous studies have been devoted to the detailed study of the spermatogenesis and oogenesis of any Indian gastropods.

Aside from their importance in the study of reproductive biology, spermatozoa are also of considerable taxonomic and phylogenic importance. Many investigations have been done about the mechanism of spermatogenesis in different species of abalones worldwide. Spermatogenesis in *H. midae* from South Africa has been investigated by Hodgson and Foster (1992). Ultrastructure of the spermatozoa of the Australian green lip abalone, *H. laevigata* and its comparison with some other haliotids has been studied with the help of Transmission Electron Microscopy by Healy *et al.* (1998). Substantial literature on all aspects of mature sperm structure, cytochemistry and biology in *Haliotis* species are available from the northern hemisphere, which includes the Japonic abalones and the North American abalones.

In Japan, most of the works have been concentrated on the study on the acrosome reaction of the abalone spermatozoa. Shiroya *et al.* (1986) investigated the localization of actin filaments in the spermatozoa of the abalone *H. discus* with the help of transmission electron microscope. Purification of the vitelline coat lysine of the abalone spermatozoa has been done by Haino-Fukushima and Usui (1986). Sakai *et*

al. (1982) studied about the fine structural changes during the acrosome reaction of the Japanese abalone *H. discus.* Ultrastructural changes of the 'truncated cone' during the acrosome reaction in Japanese abalone spermatozoa have been studied by Shiroya and Sakai (1984). Usui (1987) studied on the formation of a cylindrical structure during the acrosome reaction of abalone spermatozoa. Detailed investigation of the coiled filamentous structure, 'truncated cone' in the acrosome abalone sperm has been done by Shiroya *et al.* in 1989.

In North America, the morphology of abalone spermatozoa has been studied in detail in *H. rufescens* by Lewis *et al.* (1980). Species specificity of abalone sperm lysine has been studied by Vacquier *et al.* (1990).

The spermatogenesis of only limited species of abalones has been studied till date. But more detailed studies have been reported from other molluscan species. The ultrastructural features of the spermatogenesis were investigated in the nudibranch mollusc *Spurilla neapolitana* by Eckelberger and Eyster (1981). The ultrastructure of the spermatozoa of *Mytilus edulis* and *Mytilus galloprovincialis* has been described by Hodgson and Bernard (1986). A comparison of the structure of the spermatozoa and spermatogenesis in 16 species of patellid limpet has been studied by Hodgson and Bernard (1988). In this study, it has shown that each species has a sperm with a unique morphology, indicating that the spermatozoa can be used as a taxonomic character. Healy (1990) described the euspermatozoa and paraspermatozoa in the trochoid gastropod *Zalipais laseroni.* Ultrastructure of sperm development and mature sperm morphology of three galeommatid bivalves, *Divariscintilla yoyo*, *D. troglodytes* and *Scintilla* sp. in Eastern Florida have been described by Eckelbarger *et al.* (1990). The morphology of the mature spermatozoon

of the gastropod, *Bulinus tropicus* has been described by Brackenbury and Appleton (1991). Sousa and Olivereira (1994) studied the spermatogenesis in *Helcion pellucidus* (Gastropoda) by Transmission Electron Microscopy.

The literature on the oogenesis and the ultrastrucural details of the ovary of abalones is even scarce. Structural features of the abalone egg extracellular matrix have been studied in detail by Mozingo *et al.* (1995), in *H. rufescens*. External surface of the abalone eggs were studied using Quick-freeze deep etch technique by Shiroya and Sakai (1995). Sun *et al.* (1997) investigated the morphology and ultrastructure of the mature unfertilized and fertilized eggs of the abalone, *H. discus hannai* using Scanning and Transmission Electron Microscopy. The mechanism of oogenesis in many other molluscan species has been reported. Taylor and Anderson (1969) carried out the fine structural analysis of oogenesis in the gastropod, *Hyanassa obsoleta*. Ultrastructuaral and cytochemical analysis of oogenesis in the squid, *Loligo pealei* were done by Selman and Arnold (1977). The fine structure of the oocyte and follicle cells of *Limnaea stagnalis* has been described by Rigby (1979). Ultrastructural changes during the course of development of oocytes of the mussel, *Mytilus edulis* have been described in detail by Pipe (1987). Desilets *et al.* (1995) studied the early fertilization events in the giant scallop, *Placopecten magellanicus* using electron microscopy.

Reproductive success is closely linked to the environment. As a result variations in the reproductive cycles of marine invertebrate species often accompany change in latitude (Giese, 1959; Vernberg, 1962; Giese and Pearse, 1974; Sastry, 1970). These variations are the result of intraspecific physiological differences caused by genetic divergence, phenotypic adaptations, or a combination of both (Prosser,

1973). Newman (1967) found variations in the reproductive cycle of *H. midae* at less than 50 miles apart. Webber and Giese (1969) found different spawning times in populations of the black abalone, *H. cracherodii*, that were only 7 miles apart.

Environmental parameters that promote spawning in broadcast fertilizers are varied and, to some extent documented (Gunter, 1957; Giese, 1959; Kinne, 1963). Even less is known about the parameters that control the initiation of the gametogenesis. Newman (1967) surmised about the importance of temperature as a stimulus for gonad maturation and spawning, but cautioned that other possible influences may be involved. The importance of local environmental parameters in regulating the reproductive rhythmicity is emphasized in *H. ruber* and H. *cracherodii*, which have different spawning periods at different locations (Shepherd and Laws, 1974; Webber and Giese, 1969).

Environmental factors are probably important in affecting the active gametogenic period. Some species (eg. *H. cracherodii*) show more than one period of active gametogenesis in a reproductive cycle (Webber and Giese, 1969). They found that the eggs in that species matured only periodically in the population but the testes had mature sperms throughout the reproductive cycle. Populations not in synchrony have been reported. Young and DeMartini (1970) found all stages of gametogenesis in the gonads of *H. rufescens* collected throughout the year in California.

From the literature review it is evident that no study on the reproductive biology of abalones of Indian coast has been done. Hence an attempt was made to study the reproductive biology of *Haliotis varia* that is the only species of abalone reported from the Indian coast. The distribution of abalones along the Manauli and Pullivasal Islands in the Mandapam area of Gulf of Mannar is reported for the first time during the

present study. The main objectives of this chapter include studying the annual reproductive cycle, sex ratio, size at first maturity, fecundity, gonadal histology and mechanism of spermatogenesis and oogenesis of the tropical abalone *Haliotis varia*.

MATERIALS AND METHODS

1. COLLECTION AND TRANSPORTATION OF SPECIMENS

Monthly samples of *H. varia* were collected from the intertidal rocks of Gulf of Mannar for a period of 15 months from December 1997 to February 1999. The collection was made at two stations namely Tuticorin and Mandapam. At Tuticorin, *H. varia* is found to be attached to the granite stones along the coral bed inside the harbour basin, whereas at Mandapam station, abalones are attached to the coral stones at the 'paars' behind the Krusadi, Pullivasal and Manauli Islands. The collection was made using a chisel and care was taken not to damage the foot while dislodging from the rocks.

The collected animals were transported from Tuticorin to the Mandapam Regional Center laboratory of CMFRI by road during late hours. They were placed in a round perforated asbestos sheet (Plate III2) in a bucket along with a wet piece of gunny. To keep them moist, sea water was sprinkled on the abalones frequently during transportation. The duration of transportation was 6 to 8 hours. The transported abalones were stocked in 1.5-ton capacity FRP tank with filtered sea water

The specimens collected from 'paars' at Mandapam were transported to the laboratory by boat and they were immediately stocked in the FRP tank. The animals from both the stations were placed in the tank over night to allow the clearance of waste materials accumulated in their body.

2. MEASUREMENT OF ECOLOGICAL PARAMETERS

During each collection, atmospheric and water temperatures were recorded at the collection sites at 08.00 hours, throughout the period of investigation. Similarly, salinity, pH and dissolved oxygen were also determined by taking sea water samples from the collection site to the laboratory. The salinity determinations were done by the titration procedure as described in detail by Strickland and Parsons (1968). Salinity was calculated from the chlorinity values by the formula,

Salinity %o = 0.03+(1.8.5 X chlorinity)

pH was measured in the laboratory using a digital pH meter. The dissolved oxygen was determined by the Winkler titration method (Strickland and Parsons, 1968).

3. REPRODUCTIVE BIOLOGY

A minimum of 45 specimens monthly were taken from each station for studying the annual gonadal cycle, gametogenesis and maturity stages. The total body weight of each animal was taken to the nearest milligram (mg) using a sensitive balance. They were then measured to the nearest millimeter (mm) for shell length, shell width and shell height (Plate I) using vernier calipers. The specimens were then shucked from the shell and the gonads were examined. The soft body weight, digestive gland weight and gonad weight were also taken to the nearest milligram. Different morphological characters of the gonad such as colour, percentage of gonad in the conical appendage, gonad volume, and digestive gland volume were also noted. The gonads were assigned to six different maturity stages as suggested by Wood and Buxton (1996), based on the colour of the gonad, macroscopic appearance such as

size and shape and nature of turgidness and microscopic appearance such as ova diameter measurements and presence of sperms.

3.1. SEX RATIO

After bringing to the lab, the animals were segregated sex-wise. The total number of males and females in the sample were recorded. The sex ratio of the population was then calculated for each month.

3.2. GONADOSOMATIC INDEX

The gonadosomatic index (GSI) for each abalone was calculated using the formula (Webber and Giese, 1969).

$$GSI = \frac{\text{Wet weight of the gonad in grams}}{\text{Soft body weight of animal in grams}} \times 100$$

The range and average values of GSI were determined for each maturity stage and also for each month.

3.3. OOCYTE SIZE FREQUENCY PROFILES

Size increase of oocytes is a function of oogenesis and hence micrometric measurements of oocytes in different stages of ovarian maturation will provide an important criterion for classifying the oocytes. Oocyte diameter measurements were taken from ovaries belonging to various developmental stages and oocyte size-frequency profiles were constructed, with a view to trace development of ova from immature stage to ripe condition (Webber and Giese, 1969; Wood and Buxton, 1996). A representative piece from the fresh collected ovary was gently teased on a clean, dry glass slide along with a drop of solution of 1% formalin in 0.6% NaCl (Shehdah *et al.* 1973). Since the oocytes strongly deviate from a spherical shape, the diameter

of the egg was measured along th largest and shortest axes and the average of the two values were taken. The mea urement was taken using an ocular micrometer, which was calibrated using a stag micrometer (each ocular division was found to be equal to 0.0114 mm at a magnific ion of 100X). The measurements were classified into 10 µm class intervals and the prominent mode and the largest oocyte diameter (LOD) were measured for each ma rity stage.

3.4. FECUNDITY

The potential fecundity or t e number of eggs available to be spawned in a single spawning was estimated fo abalone in stage III (ripe) of ovary development when the ovary contains exclusive ripe oocytes. These estimates are based on sub sampling of unbiased samples of varies from gravid abalone collected during the peak spawning period, as recomm nded by Newman (1967).

Subsamples weighing abo 100 mg were taken from the anterior and the middle regions of the conical shap d, pre-weighed ripe ovary and placed in a known volume of Guilsion's fluid to g the eggs free from the ovarian trabeculae. Subsamples were taken from the bove solution with the eggs and the numbers of yolk eggs were counted under a c ssection microscope in a counting chamber. The fecundity of the abalone was deter ined using the formula

$$F = \frac{nG}{g}$$

Where, F = Fecundity

n = Number o eggs in the subsample

G = Total weig t of the ovary in grams

g = Weight of he subsample in grams

The relationship between t fecundity (F) and the shell length (L), fecundity and total body weight (W) and the cundity and total gonad weight (G) of the abalone were determined using regression quations.

3.5. SIZE AT FIRST SEXUAL MA IRITY

In order to ascertain the si at which abalones attain first sexual maturity, a series of young abalones ranging om 6 mm in shell length onwards were collected during the peak of breeding seas and their gonad developments were examined morphologically and microscopica . The measurements were grouped into 2 mm length intervals and the percentag of individuals with mature gonads was calculated against each of the class interval o estimate the minimum size of maturity at 50% level (Shepherd and Laws, 1974; T tschulte and Connell, 1981).

3.6. HEPATOSOMATIC INDEX

The hepatosomatic index (H I) was calculated using the formula suggested by Crupkin *et al.* (1988).

$$HSI = \frac{\text{Wet weight of whole dig tive gland in grams}}{\text{Wet soft body weight abalone in grams}} \times 100$$

The range and average va es of hepatic index at each maturity stage was calculated.

4. HISTOLOGY OF GONAD

4.1. LIGHT MICROSCOPIC STUDIE

The histological changes t ing place in the male and female gonads of *H. varia* during various stages of mat ity were studied. Sample pieces of gonad (5 mm thick) belonging to different maturi stages, were taken from freshly killed specimens

and fixed in 10% neutral buffere formalin for 20-24 hours. The tissue was then thoroughly washed with running fre h water for 6-7 hours and then stored in 70% ethyl alcohol until further processing. code number was given to each tissue and its details recorded. The tissues w e then dehydrated in graded alcohol series by following the standard procedure (eesner, 1960). These tissues were then cleared with chloroform/ xylene for 3-4 hou , impregnated with and embedded in paraffin wax containing ceresin (Glaxo, melting oint 58-60 ºC).

The prepared blocks were en trimmed, catalogued and stored in polythene bags. Sections of 5-7 µm thickn ss were cut manually using a rotary microtome. Mayer's egg albumin: glycerol (1: v/v) (Gray, 1973) was used as the adhesive for fixing the paraffin ribbons with s ctions, on clean dry glass slides. The slides containing the spread ribbons were hen incubated for few minutes at 40 ºC on a slide warmer. The sections were dep raffinised, hydrated and stained with Ehrlrich's haemetoxylin (Weesner, 1960), Ha i's haemetoxylin (Preece, 1972) and eosin as the counter-stain. DPX was used as th mounting medium for all the slides.

The sections were observe and photographed using a binocular compound microscope (Nikon, Type 104, Ja an) with a camera unit. Appropriate projection eyepiece was used and the photo raphs were taken using 24 X 36 mm NOVA (125 ASA), black and white negative fi n. The prints were taken on soft, glossy, single weight special paper and enlarged o the required size.

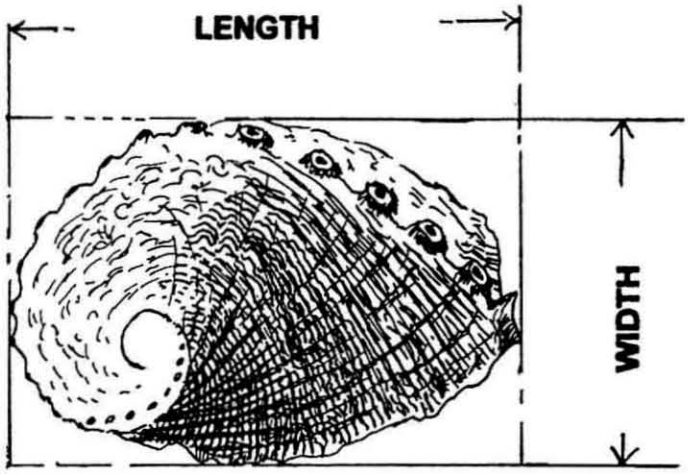
4.2. ULTRASTRUCTURE STUDIES Transmission Electron Microscopy-TEM):

The ultrastructural details the oogenesis and the spermatogenesis were studied with the help of transmiss on electron microscope (TEM). For this the male and female gonadal tissues exci d at different maturity stages were used. The

tissues were removed from the digestive gland and placed in fresh primary fixative of 3% gluteraldehyde buffered with 0.2 M cacodylate buffer containing 2% tannic acid and 6% glucose at pH 7.2, for 3 hours at 4 ºC. After primary fixation tissues were washed three times in cacodylate buffer, each about 15 minutes duration and post fixed in 1% buffered Osmium tetroxide for about 1 hour at 4 ºC. Properly fixed tissues were again washed in cacodylate buffer and then dehydrated in a graded acetone series. The tissues were infiltrated and embedded in a low viscosity resin-embedding medium (Spurr, 1969) and kept at 60 ºC for 36 hrs.

After proper trimming, ultrathin sections were cut from the polymerized blocks with a glass knife on an ultramictotome, 'LKB BROMA'. Ultra thin sections (300-600 Aº) collected on uncoated copper grids (300 mesh), were post stained in 1% Uranyl acetate in methanol (Watson, 1968) and Lead citrate (Reynolds, 1963). After drying the grids were examined with a 'Hitachi 600' electron microscope at 50 Kv. Ultra structural details of the various stages of male and female gonads were examined and relevant areas were photographed using 'Graphic Kodak lith' film.

PLATE I



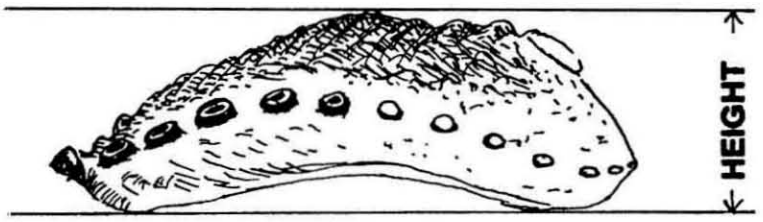


Plate. Diagram of abalone shells showing various measurements

PLATE II

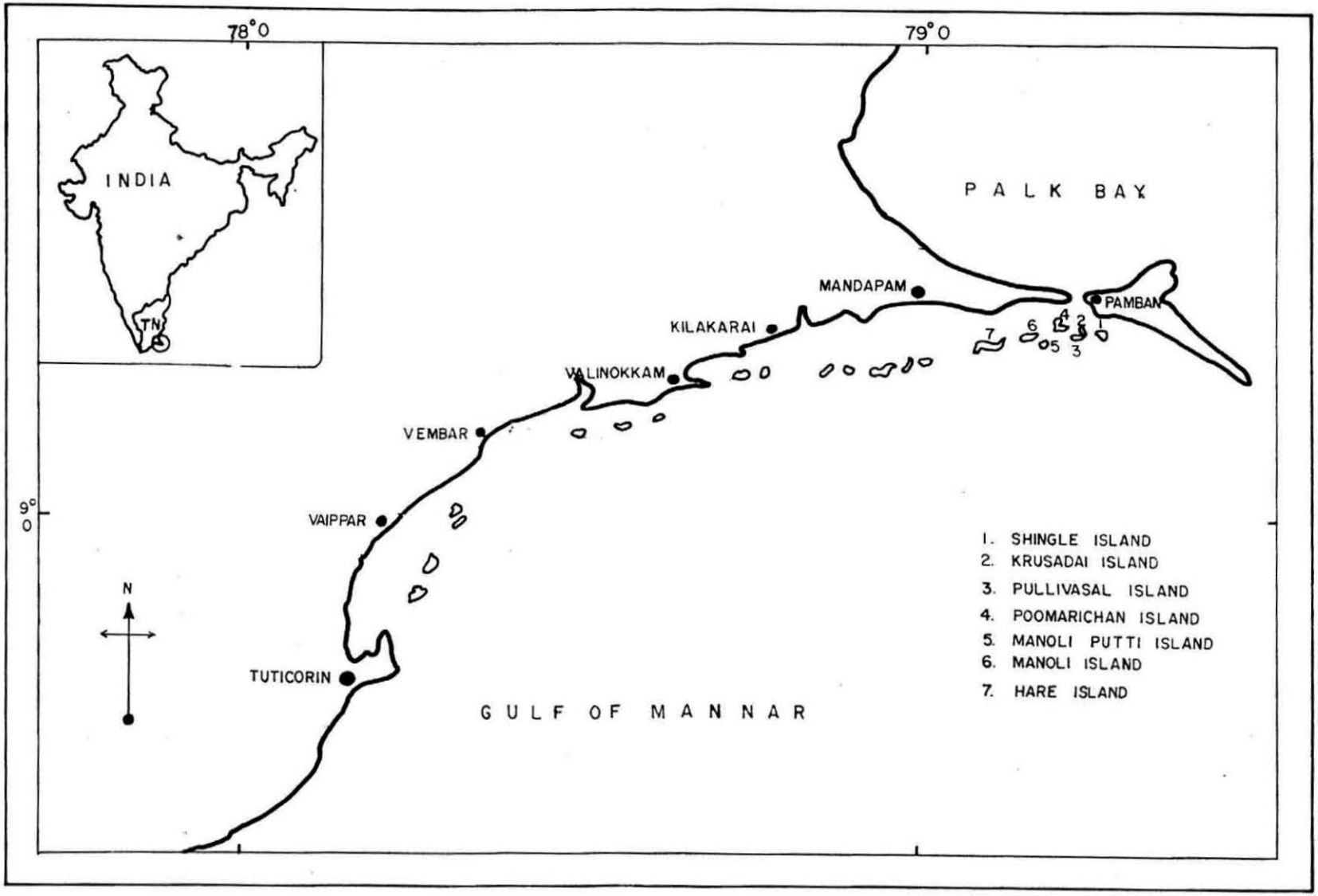
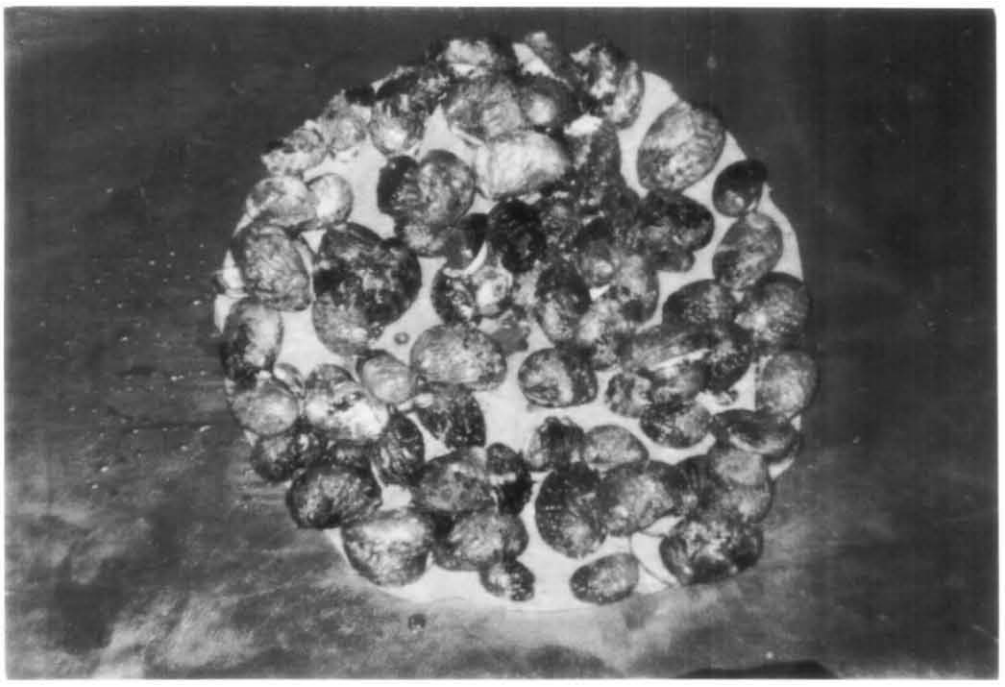


Plate. East coast of Southern Tamil Nadu showing the study areas (Tuticorin and Mandapam)

PLATE III

1. INSAT 1B Picture of Southern parts of Tamil Nadu and Gulf of Mannar showing the collection sites (a:Tuticorin, b: Mandapam)



2. Collected abalones kept on asbestos sheet for transportation

PLATE IV

1. A view of Tuticorin Harbour collection site

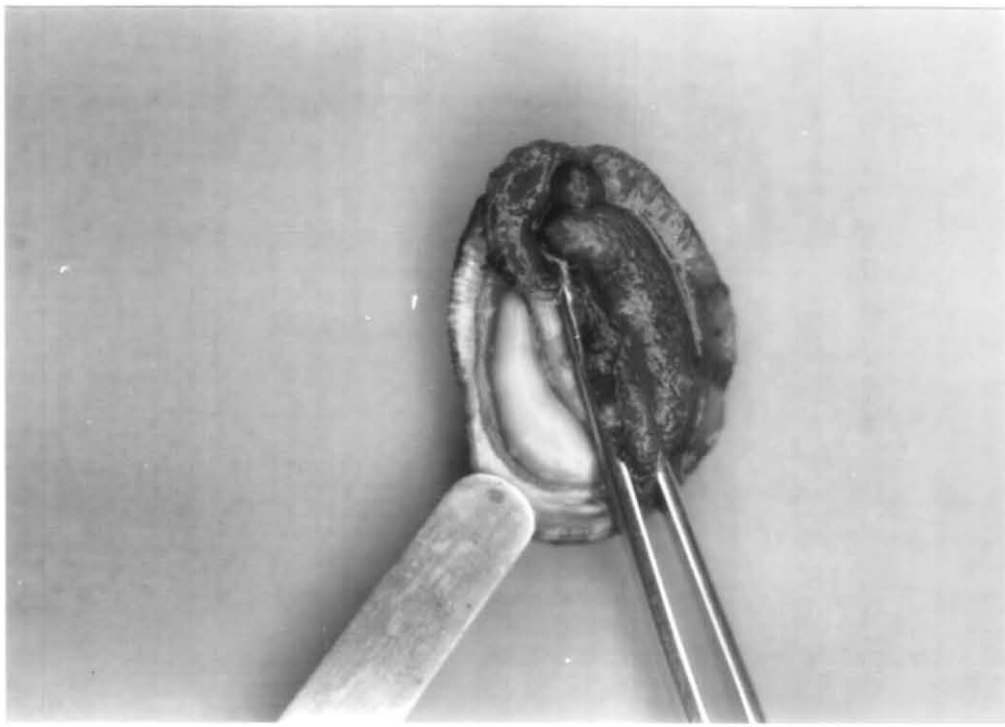
2. A view of Mandapam 'paars' collection site

PLATE V

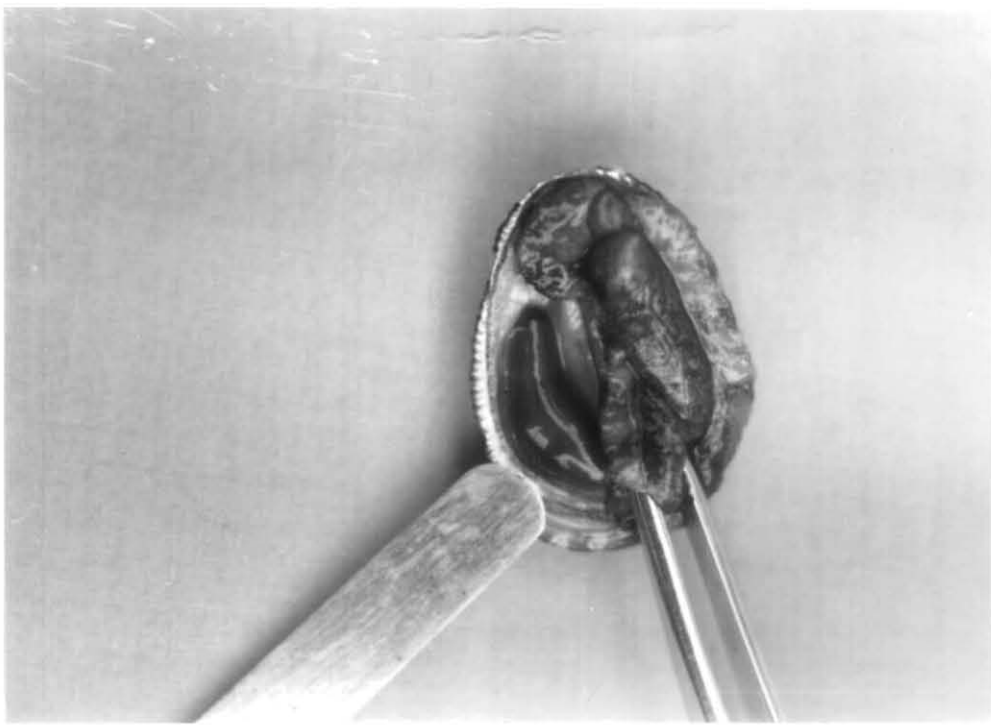
1. Adult *Haliotis varia* from Tuticorin station

2. Adult *Haliotis varia* from Mandapam station

PLATE VI

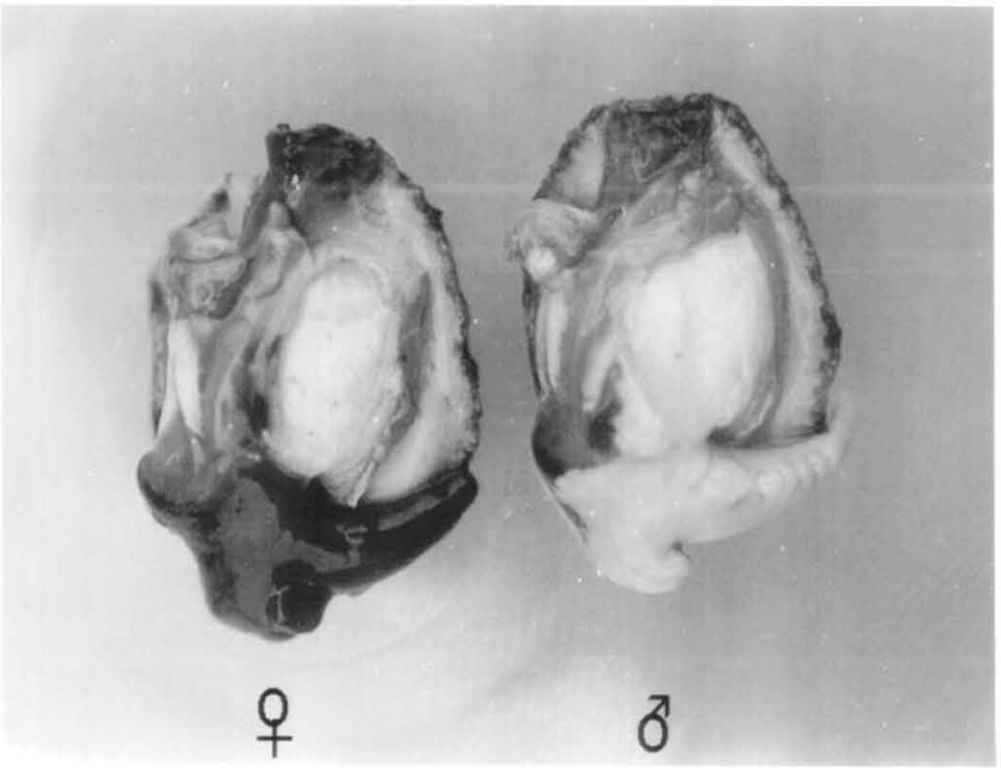


1. Exposed ripe male gonad of *Haliotis varia*

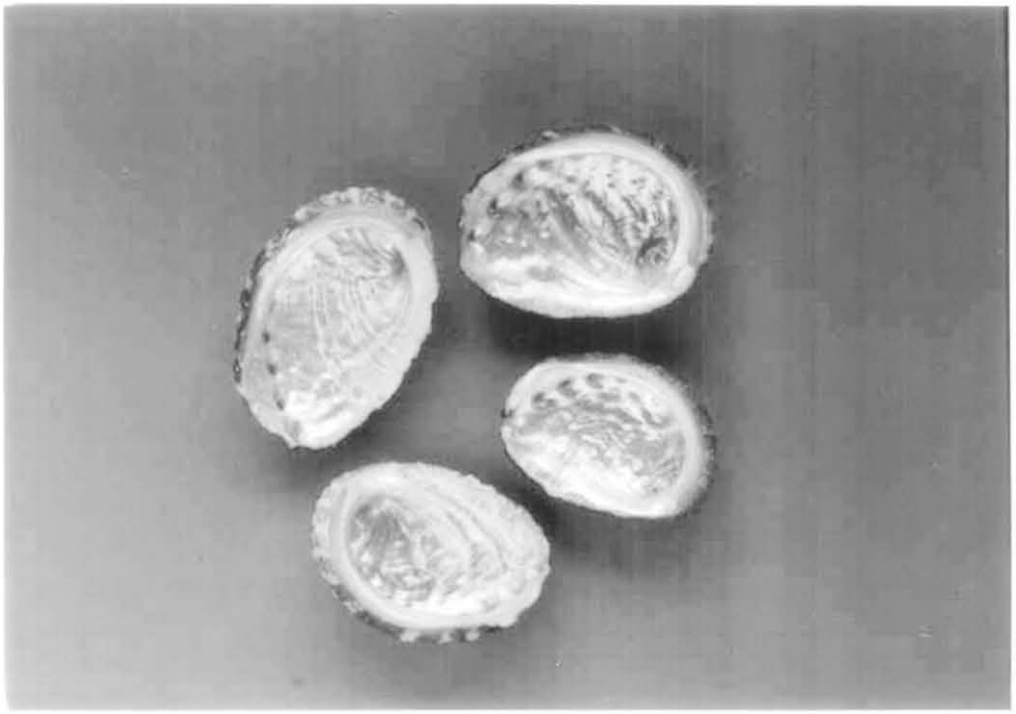


2. Exposed ripe female gonad of *Haliotis varia*

PLATE VII



1. Dorsal view of adult *Haliotis varia* removed from the shells showing ripe male and female gonads



2. Shells of *Haliotis varia* showing the pearly interior

RESULTS

1. DESCRIPTION OF THE GONAD

Haliotis varia is dioecious and the gonad lies over part of the stomach and surrounds the digestive gland. Abalone gonad can be viewed externally without shucking the animal, pulling the mantle by a needle. The gonad and digestive gland together with a part of stomach form the conical appendage. The gonad is located between the outer epithelial layer of the conical appendage and the digestive gland. The gonad consists of a large lumen, which is bounded by germinal epithelium with a connective tissue base. The outer gonad wall is similar in both sexes and consists of an epidermis, which is a simple, glandular, columnar epithelium covered with a thin layer of cuticle. A fibro muscular layer composed of connective tissue and muscle fibers lies beneath the epithelial layer. The coelomic epithelium together with strands of underlying connective tissue grows outward in thin folds that form trabeculae between the overlying connective tissue of the digestive gland and the integument.

The lumen of the testis is crossed by tubes of connective tissue, which are normally vertical in the well-developed testis. The connective tissue tubes are surrounded by a cuboidal germinal epithelium, similar to that of the walls of the testis. The arrangement of these tubes, which run vertically between the inner and outer testis walls, is shown in Plate VI5, a photomicrograph of a well-developed testis sectioned horizontally. When fully mature, the whole testis lumen is packed with sperms. The germinal epithelium in the lumen gives rise to spermatogonia of approximately 5.33 μm diameter, which develops into primary and secondary

spermatocytes of 4 to 4.23 μm diameter and highly basophilic spermatids of 1.23 to 3.43 μm diameter. Final maturation is into spermatozoa, which consists of an anterior oesnophilic acrosome followed by a posterior elongated basophilic nucleus of 2.8 μm diameter excluding the flagellum. Condensed mitochondria form the middle piece of the spermatozoa. The tail or flagellum of mature sperm was barely visible, even under high magnification.

The ovarian lumen is filled with trabeculae of connective tissue and muscle fibers supporting the germinal epithelium. The trabeculae are arranged parallel to one another in a series of polyhedral columns, except near the digestive gland, projecting from the outer ovarian wall towards the digestive gland. Some of the trabeculae attach to the inner ovarian wall, indicating that they have a supportive role as well as acting as a base for germinal tissue. Nearer the digestive gland, the eggs are less closely packed than those near the integument and their shape is less distorted. In the ripe ovary, most trabeculae end freely in lumen, as in *H. midae*, perhaps allowing for free movement of eggs during spawning (Newman, 1967). Branches of hepatogenital blood vessels are found in the connective tissue of the inner ovarian wall and blood sinuses project in to the ovarian wall. The oogonia arise from the germinal epithelium and give rise to primary oocytes. The process of vitellogenesis starts when the primary oocytes reach 50 μm in diameter.

2. CLASSIFICATION OF MATURITY STAGES OF GONAD

Based on the size, shape, color, and texture of the gonad and microscopic structure through histological examination, six arbitrary stages of maturity in male and female *H. varia* have been identified as suggested by Wood and Buxton (1996).

2.1. MALE

Stage I : Early maturing

The testis in this stage is flaccid and pale orange in colour. It comprises only 40% of the conical appendage. In cross-section, gonad appears as a thin sheath over the thick digestive gland. The lumen of the testis is traversed by branching tubes of connective tissue (Plate VIII1). The germinal epithelium in the lumen gives rise to spermatogonia of approximately 5.33 μm diameter, which develops into primary and secondary spermatocytes of diameter 4.23 and 4 μm respectively. The testis mainly contains spermatogonial cells and spermatocytes. It was observed that the spermatogonial cells and spermatocytes are arranged in a graded manner in the testis so that the growing spermatocytes were shifted to the interior of the testis (Plate VIII4). The spermatids and sperms are seen in the lumen near the digestive gland and lesser in number.

Stage II : Late maturing

Gonad becomes thicker and the colour changes to bright orange. Gonad comprises about 60% of the conical appendage. Proliferation of spermatogonial cells from the germinal epithelium of the lumen continues (Plate VIII2). The testis in this phase contains large number of spermatocytes and spermatids. Few sperms are seen with their tails facing towards the digestive gland. The spermatocytes are abundant near the walls of the testis and around the tubules. Spermatids are highly basophilic and have a diameter of 1.23 to 1.43 μm.

Stage III : Ripe

The ripe male gonad is turgid, cylindrical and bright cream in colour. It comprises 80-90% of the conical appendage (Plate VII1). A ripe testis makes up 15-

20% of the soft body weight of *H. varia*. Little or no digestive gland area can be seen in the cross section of the conical appendage at this stage. The lumen of the ripe testis is traversed by connective tissue tubes, which are vertical in cross-section (Plate VIII5). Radially arranged spermatozoa with their tails oriented towards the digestive gland are seen plenty in the ripe testis section (Plate VIII6). As the volume of the spermatozoa increases, there is a corresponding decrease in the number of immature germ cells.

Stage IV : Partially spawned

The gonad of the post-spawned male individual is characterized by the shrinkage of gonad trabeculae. It is bright cream, flaccid and loose in appearance. Gonad comprises about 80% of the conical appendage. It is longer but not much thick. As the sperms from the lumen are ejected during spawning, the connective tissue layer increases in size (Plate IX2). Some spermatogonial cells and spermatocytes appear in the periphery of the testis. The presence of phagocytic cells in the inter trabecular spaces are noticeable. The connective tissue tubes also increase in volume.

Stage V : Spent

The spent male gonad is a thin gray sheath and flaccid in nature. The digestive gland area increases in cross-section than the gonad area. The whole organ is considerably reduced in size and within the lumen relatively few darkly staining sperm occur, presumably a residue from the previous spawning. The connective tissue tubes are more convoluted due to the collapsed testis (Plate IX3).

2.2. FEMALE

Stage I : Early maturing/ Recovering spent

Gonad appears as a thin grayish sheath over the digestive gland. Gonad comprises approximately 40% of the conical appendage. It is somewhat flabby. The cells of the germinal epithelium are easily seen and are about 6 μm in diameter. Some oocytes are seen attached to the follicle cells by a stalk, with diameters of approximately 45 μm (Plate IX4). These have well defined oolemma and prominent nuclei, for the egg is not yet filled with yolk. The oocytes under magnification appear round, sometimes irregular and transparent. Diameter of the oocytes ranges from 10 μm to 57 μm with a mode at 45 μm.

Stage II : Late maturing

The gonad becomes bluish in color and appears as a thick sheath over the digestive gland. Gonad area increases in the cross-section of the conical appendage. Large vitellogenic cells, mostly attached to the trabeculae by stalks and oriented towards the digestive gland, can be seen in plenty. In the oocytes, the basophilic nucleus migrates to the animal pole opposite to the vegetative stalk, and cytoplasm becomes oesnophilic as vitellogenesis is initiated (Plate IX6). Oocyte diameter ranges from 50 to 140 μm with a mode at 68 μm. Vitelline layer is seen around the oolemma of the oocyte.

Stage III : Ripe

The ripe ovary is blue or bluish green in colour, massive and turgid. Little or no digestive gland area can be seen in the cross-section of the conical appendage. The gonad covers around 80% of the conical appendage and becomes more cylindrical (Plate VII1). The lumen of the ovary is filled with large eggs, which have a maximum

diameter of 228 μm (Plate X1). The ripe egg is rounded to oval in shape. Most of the eggs are polymodal in shape, with a distinct nucleus and a round nucleolus (Plate X2). In the ripe ovary the number of detached oocytes is greater than the number of attached oocytes. Within the ovary parallely arranged trabeculae, with one end attached to the inner ovarian wall and the other end free in the lumen can be seen. The eggs are loosely packed in the lumen near the digestive gland. The diameter of the ripe eggs ranges from 140 to 228 μm with a prominent mode at 171 μm.

Stage IV : Partially spawned

Gonad is flabby and thick in consistency. Colour is still blue with loosely packed eggs. Gonad comprises 80% of the conical appendage. In the lumen near the digestive gland, the eggs are very less in number, compared to the ripe ovary (Plate X3). Some oocytes can be seen in a state of reabsorption. The oocyte diameter ranges from 140 to 228 μm. Presence of phagocytes is observed in this stage.

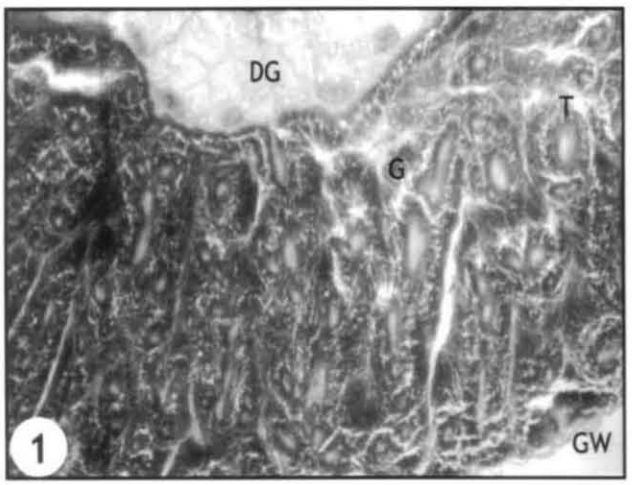
Stage V : Spent

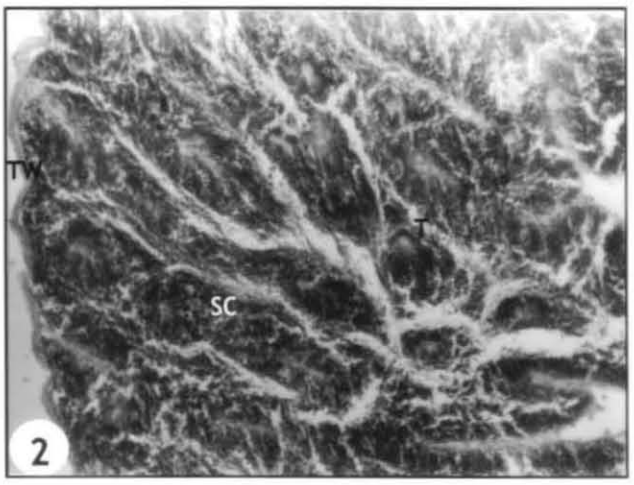
The highly shrunken and collapsed gonad covers the digestive gland as a thin translucent and flaccid sheath, loosely packed with some primary oocytes. The ovary is considerably reduced in size and the trabeculae are inclined due to the collapsed state of the lumen (Plate X4). Lacunae are present in the trabeculae, and within the lacunae amoebocytes are often found. A few opaque, large disintegrated oocytes are observed as scattered which undergo the process of resorption.

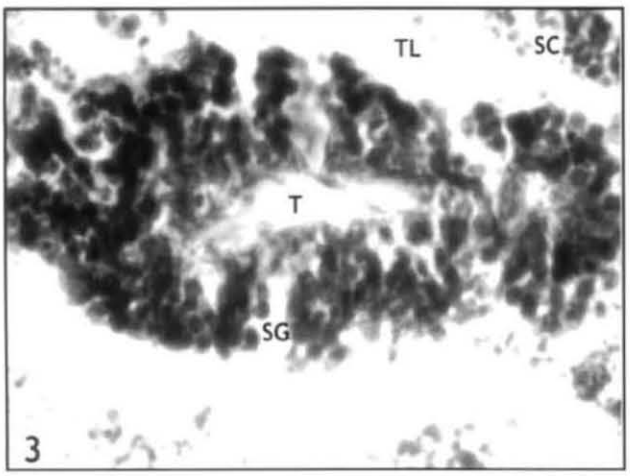
Stage VI : Indeterminable stage

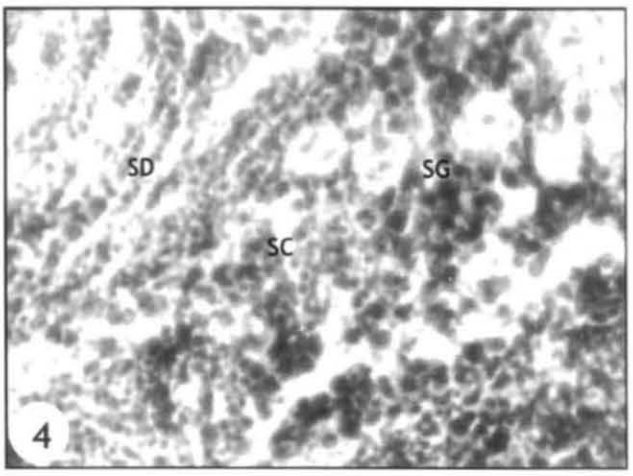
In the early developmental stage, gonads of male and female abalones are indistinguishable even in histological examination. And also, following the spent

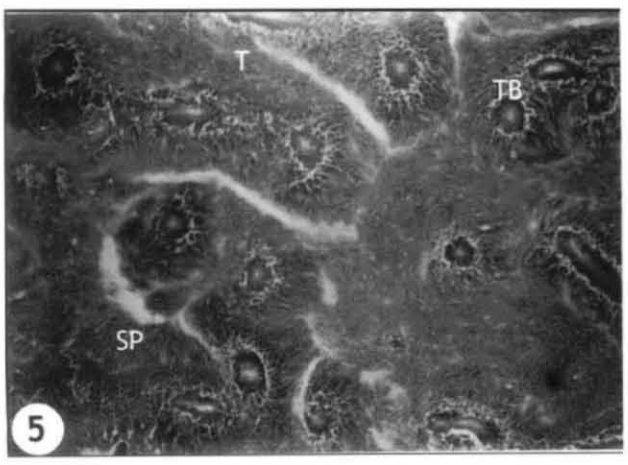
PLATE VIII











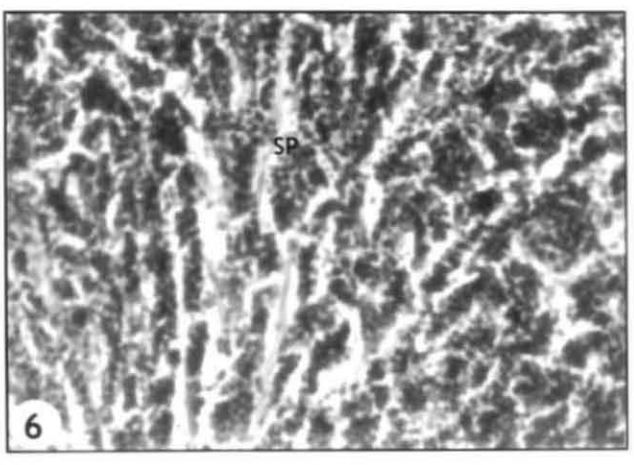


PLATE IX

1. Section through a ripe testis showing spermatozoa and connective tissue tubules (SP- Spermatozoa, TB- Tubule, TW- Testis wall). X100.

2. Light micrograph of a partially spawned testis with collapsed trabeculae (T- Testis, TB- Trabeculae, TW- Testis wall). X100.

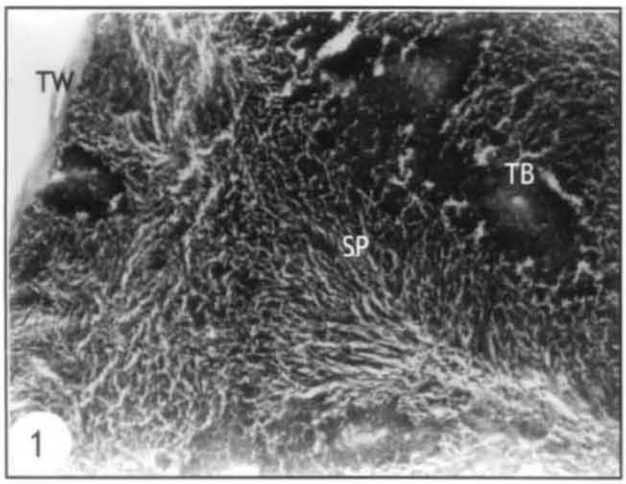
3. Section through a spent stage testis with highly collapsed trabeculae (SP- Unspawned spermatozoa, TB- Trabeculae). X100.

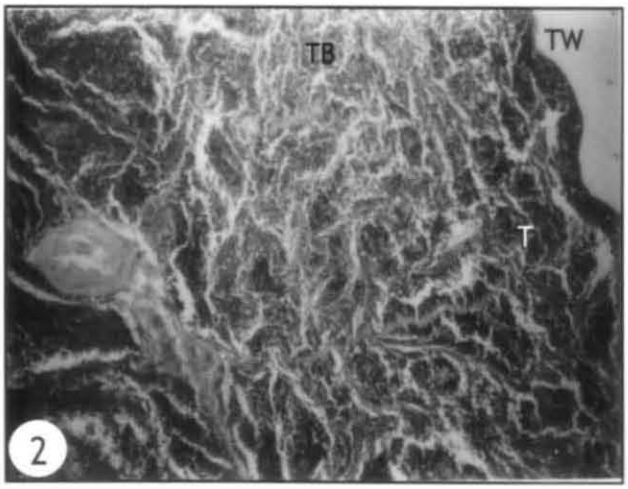
4. Light micrograph of an early maturing ovary (DG- Digestive gland, OW- Ovary wall, PO- Primary oocytes, TB- Trabeculae) X100.

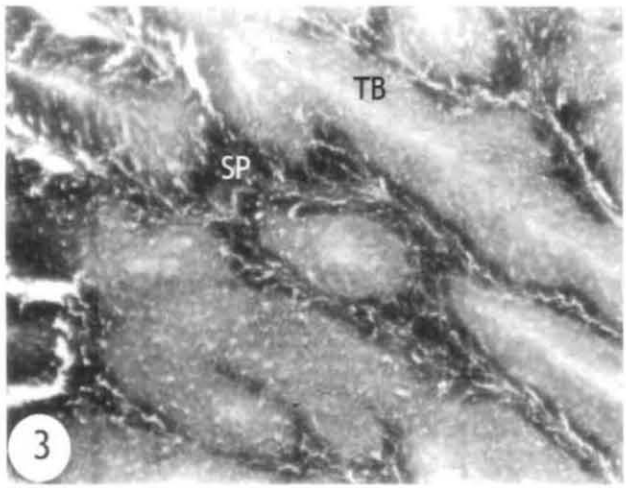
5. Light micrograph of the early maturing ovary showing stalked primary oocytes (OG- Oogonia, OW- Ovary wall, PO- Primary oocytes, TB- Trabeculae). X200.

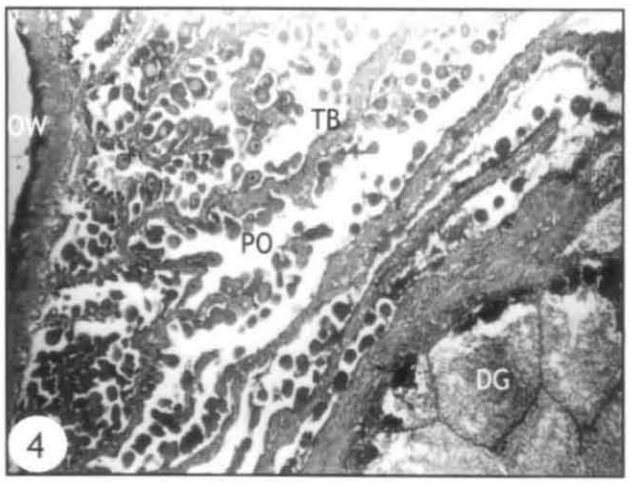
6. Section through a late maturing ovary showing vitellogenic oocytes (PO- Primary oocyte, OW- Ovary wall, VO- Vitellogenic oocyte, Y- Yolk granules). X200.

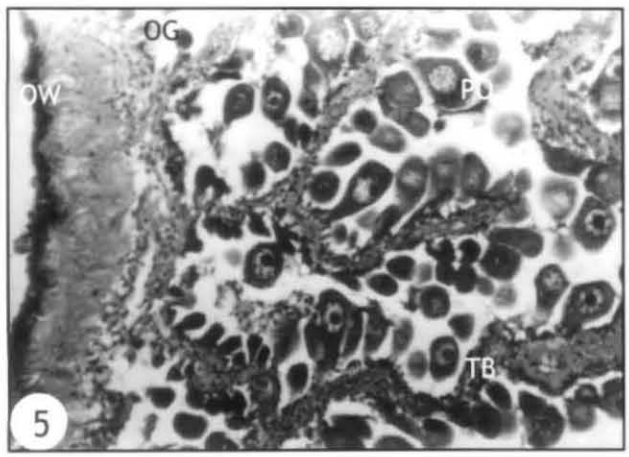
PLATE IX











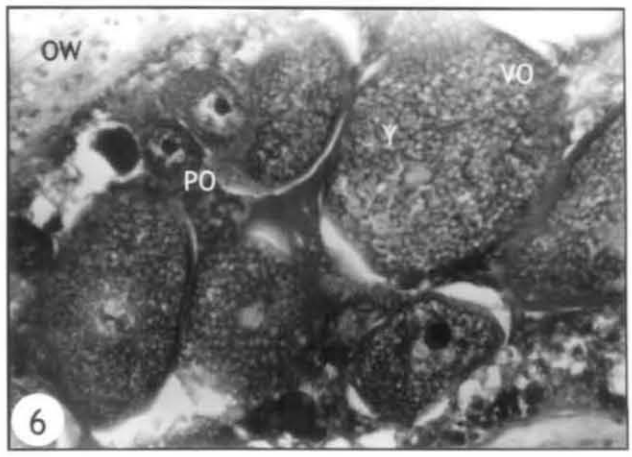


PLATE X

1. Light micrograph of a ripe ovary with exclusively packed ripe oocytes (RO- Ripe oocytes, PO- Primary oocytes, N- Nucleus). X40.

2. Section through a ripe ovary showing the structure of ripe oocytes (RO- Ripe oocyte, N- Nucleus, NL- Nucleolus, Y- Yolk, VM- Vitelline membrane). X200.

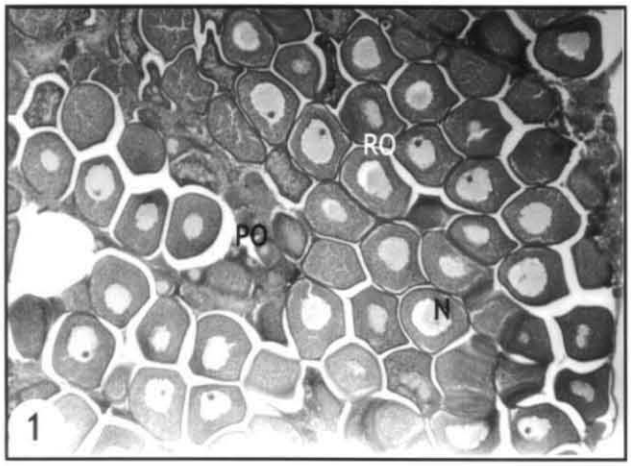
3. Light micrograph of partially spawned ovary with loosely packed oocytes (OL- Ovarian lumen, RO- Ripe oocytes, TB- Trabeculae). X40.

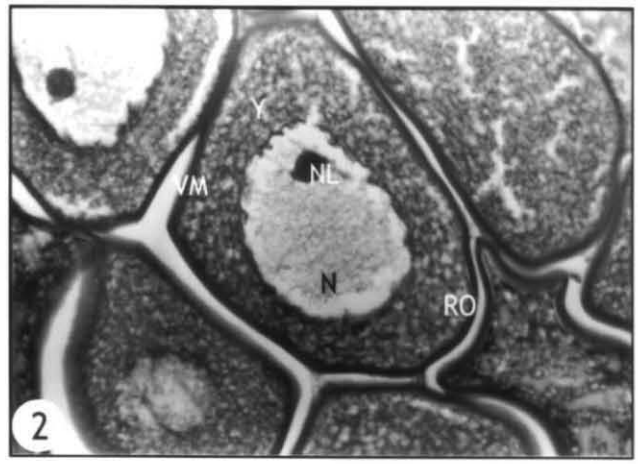
4. Section through a spent ovary showing the collapsed lumen (OL- Ovary lumen, PO- Primary oocytes, TB- Trabeculae, UO- Unspawned oocytes). X100.

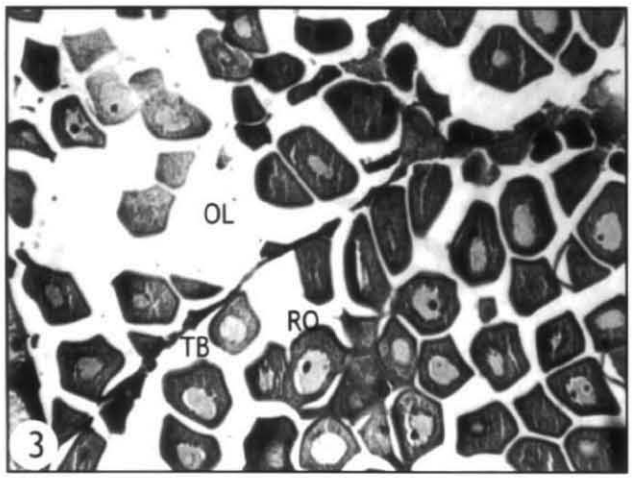
5. Light micrograph of a recovering spent ovary with developing oocytes (OL- Ovarian lumen, PO- Primary oocytes, TB- Trabecuale, URO- Unspawned ripe oocyte). X100.

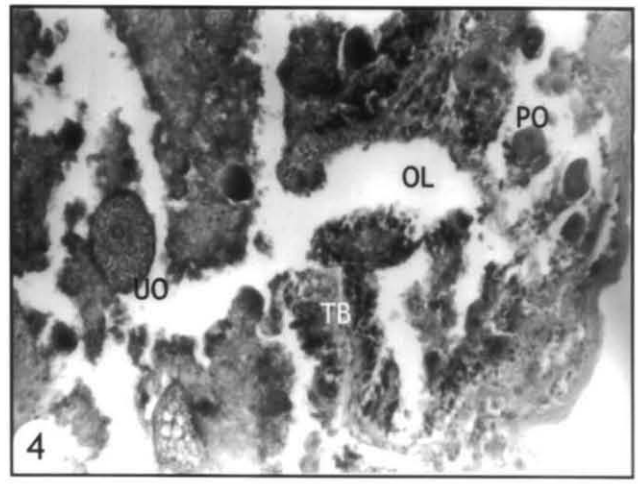
6. Section through an indeterminate stage gonad with more digestive gland area (DG- Digestive gland, G- Gonad, GW- Gonad wall). X100.

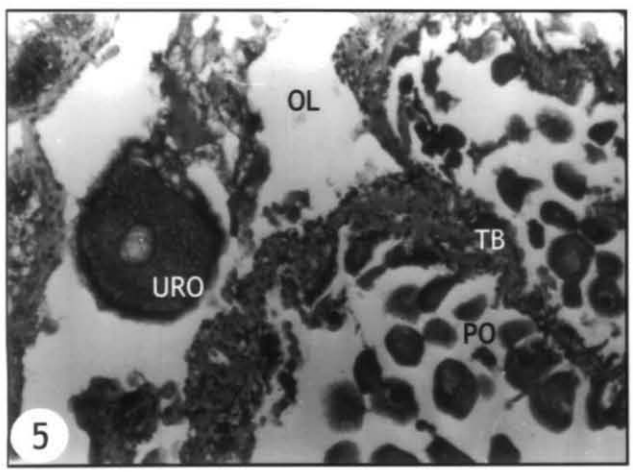
PLATE X

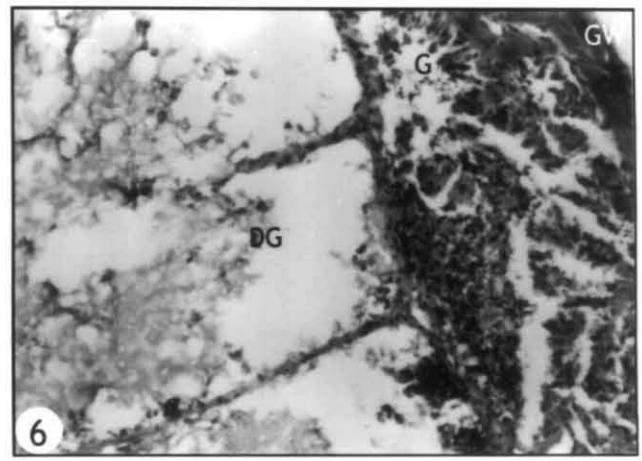












phase the male and female gonads enter into a short quiescent phase, in which the sexual identity is totally lost. The indeterminable gonad of *H.varia* is dark, grayish thin sheath comprising 30% of the conical appendage. Gonad lumen is almost filled with branches of trabeculae, which is attached to both the outer gonadal wall and the digestive gland (Plate X6).

3. REPRODUCTIVE BIOLOGY

3.1. Gonadosomatic Index

The Gonadosomatic index (GSI) during different maturity stages for female and male *H. varia* and monthly mean GSI from Tuticorin and Mandapam stations were calculated. In the Tuticorin station, the mean GSI for males was found to be 3.29 in stage I, which showed a gradual increase to 5.11 in stage II. The highest value of GSI, 11.03 was observed in stage III followed by a steep decline in the spent stage (stage V) with a mean value of 4.25. In the female specimens the highest GSI observed was lesser than that in the male, which was 10.64 in stage III. The GSI for the stage I was 3.11 and that for stage V was lesser with a mean value of 2.75. The least value of GSI observed was in the stage VI with a mean value of 1.99.

In the Mandapam station, for males, the highest value observed in stage III was 8.42 with low values of 3.40 and 3.99 in stages I and V respectively. For the females, the GSI was found to be 3.13 in stage I with a gradual increase to 3.99 in stage II. The highest value observed was in stage III with a mean value of 8.21. The value for Indeterminable stage (stage VI) was found to be 3.18. The variations in the gonadosomatic index in Tuticorin and Mandapam stations are shown in tables 1 to 6 and drawn in figures 1 to 6.

Table 1. Variations in the gonadosomatic index (GSI) and hepatosomatic index (HSI) in the different maturity stages of male and female *H. varia* at Tuticorin station

Maturity stages	GSI		HSI	
	Male	Female	Male	Female
Stage I	3.290 ± 1.664	3.119 ± 1.687	4.438 ± 1.355	6.710 ± 1.265
Stage II	5.110 ± 1.812	4.071 ± 2.596	4.515 ± 1.058	4.666 ± 2.145
Stage III	11.03 ± 3.971	10.64 ± 3.286	3.550 ± 1.896	2.973 ± 4.233
Stage IV	6.088 ± 2.592	5.298 ± 1.986	5.085 ± 1.631	4.231 ± 0.655
Stage V	4.253 ± 2.365	2.755 ± 0.830	4.944 ± 2.353	4.634 ± 1.427
Stage VI	1.991 ± 0.948		2.842 ± 0.925	

Values Mean ± Standard deviation

Table 2. Variations in the gonadosomatic index (GSI) and hepatosomatic index (HSI) in the different maturity stages of male and female *H. varia* at Mandapam station.

Maturity stages	GSI		HSI	
	Male	Female	Male	Female
Stage I	3.402 ± 0.942	3.135 ± 1.819	4.482 ± 2.216	4.651 ± 1.934
Stage II	5.055 ± 1.420	3.990 ± 0.899	4.979 ± 1.833	5.384 ± 2.371
Stage III	8.419 ± 1.850	8.215 ± 3.324	4.912 ± 1.517	3.713 ± 1.182
Stage IV	6.626 ± 2.002	5.193 ± 2.091	5.497 ± 2.491	4.562 ± 2.155
Stage V	3.996 ± 1.514	4.160 ± 1.306	5.837 ± 2.766	4.565 ± 0.904
Stage VI	3.180 ± 1.697		3.881 ± 2.883	

Values Mean ± Standard deviation

Table 3. Variations in the gonadosomatic index (GSI) and hepatosomatic index (HSI) of *H. varia* during the study period at Tuticorin station

Maturity stages	GSI	HSI
December '97	3.329 ± 2.00	5.124 ± 1.95
January '98	5.093 ± 3.42	3.609 ± 1.39
February	8.974 ± 5.04	4.531 ± 0.99
March	7.362 ± 4.19	3.920 ± 1.15
April	6.285 ± 3.27	4.218 ± 1.19
May	6.482 ± 3.17	3.608 ± 0.87
June	3.419 ± 1.49	3.595 ± 1.40
July	5.360 ± 4.14	7.145 ± 2.69
August	2.780 ± 1.10	3.459 ± 1.24
September	5.260 ± 2.81	4.595 ± 0.73
October	3.159 ± 4.30	4.305 ± 2.03
November	3.820 ± 1.57	5.125 ± 1.03
December	4.718 ± 2.45	5.453 ± 1.75
January '99	12.335 ± 4.30	2.154 ± 2.87
February	10.847 ± 2.78	4.920 ± 1.50

Values Mean ± Standard deviation

Table 4. Variations in the gonadosomatic index (GSI) and hepatosomatic index (HSI) of *H. varia* during the study period at Mandapam station

Maturity stages	GSI	HSI
December	6.944 ± 3.01	4.760 ± 1.69
January	6.547 ± 2.67	4.078 ± 1.68
February	4.060 ± 1.85	3.076 ± 1.13
March	3.475 ± 1.52	3.471 ± 1.07
April	6.066 ± 2.85	5.703 ± 1.97
June	4.265 ± 1.70	4.820 ± 0.77
July	4.872 ± 2.33	4.139 ± 0.87
August	3.254 ± 1.87	9.097 ± 2.18
October	3.274 ± 1.43	4.176 ± 1.41
November	4.337 ± 2.28	5.124 ± 1.02
December	6.206 ± 3.05	4.528 ± 1.33
January	7.160 ± 2.40	4.754 ± 2.17
February	5.882 ± 3.26	4.514 ± 1.83

Values Mean ± Standard deviation

Table 5. Variations in the Gonadosomatic Index (GSI) of male and female *H. varia* during the study period at Tuticorin station.

Month	Gonadosomatic index (GSI)	
	Male	Female
December '97	3.914 ± 2.12	2.659 ± 1.60
January '98	7.048 ± 3.24	4.050 ± 3.21
February	9.300 ± 3.87	7.966 ± 3.95
March	8.795 ± 3.85	6.460 ± 3.20
April	7.442 ± 3.29	4.498 ± 2.38
May	7.851 ± 3.59	5.113 ± 1.91
June	4.565 ± 1.60	2.770 ± 0.64
July	5.365 ± 3.21	5.764 ± 4.66
August	3.419 ± 1.28	2.767 ± 0.57
September	5.890 ± 2.79	4.176 ± 2.62
October	3.590 ± 1.42	2.918 ± 1.02
November	3.716 ± 1.83	3.939 ± 1.26
December	5.767 ± 2.48	2.905 ± 0.85
January '99	14.434 ± 3.46	11.244 ± 4.38
February	12.938 ± 2.29	9.454 ± 2.22

Values Mean ± Standard deviation

Table 6. Variations in the Gonadosomatic Index (GSI) of male and female *H. varia* during the study period at Mandapam station.

Month	Gonadosomatic index (GSI)	
	Male	Female
December '97	7.412 ± 2.68	6.476 ± 2.43
January '98	7.072 ± 2.26	5.596 ± 3.79
February	4.384 ± 2.30	4.341 ± 0.87
March	4.225 ± 1.74	2.517 ± 0.62
April	7.020 ± 2.58	4.976 ± 2.83
June	5.035 ± 1.81	3.454 ± 1.11
July	6.015 ± 1.87	4.123 ± 2.22
August	4.795 ± 1.94	2.169 ± 0.78
October	3.952 ± 1.13	2.716 ± 1.31
November	4.395 ± 2.31	4.262 ± 2.15
December	6.315 ± 2.01	6.051 ± 2.12
January '99	7.336 ± 1.98	6.889 ± 1.21
February	6.215 ± 2.86	5.549 ± 1.87

Values Mean ± Standard deviation

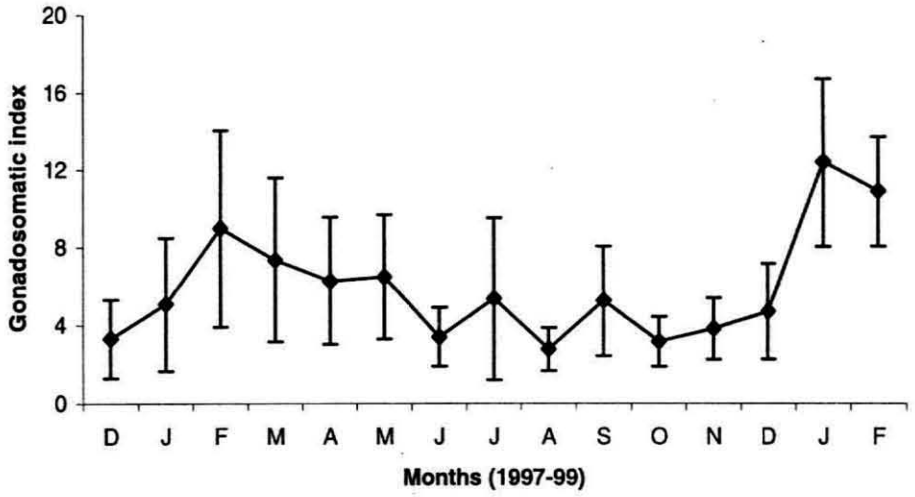


Fig.1. Monthly variation in the mean gonadosomatic index at Tuticorin station during the study period. Vertical bars represent standard deviations.

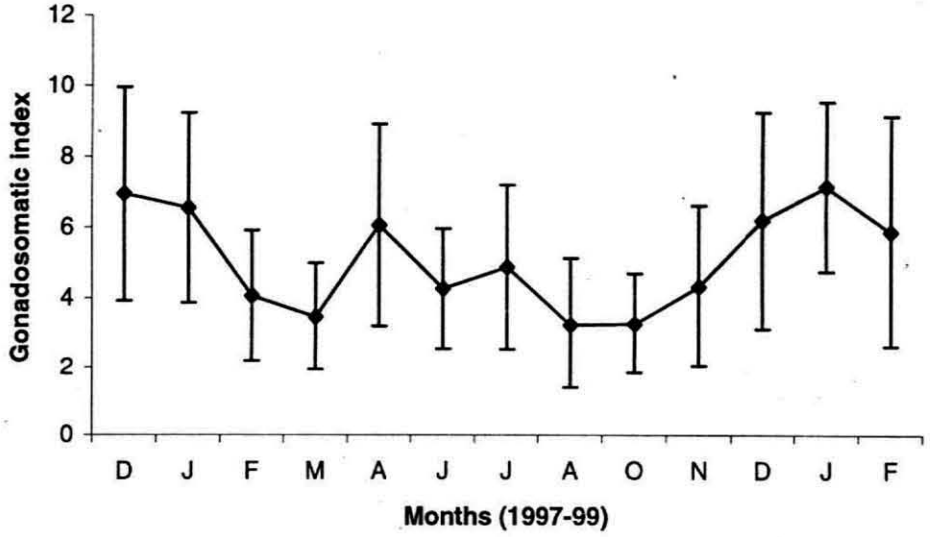


Fig. 2. Monthly variation in the mean gonadosomatic index at Mandapam station during the study period. Vertical bars represent standard deviations

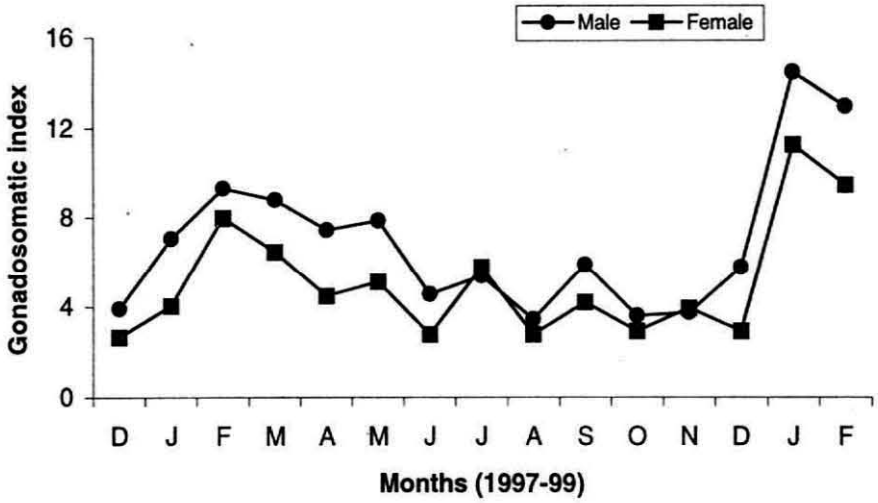


Fig.3. Monthly variations in mean gonadosomatic indices of male and female *H. varia* at Tuticorin station during the study period.

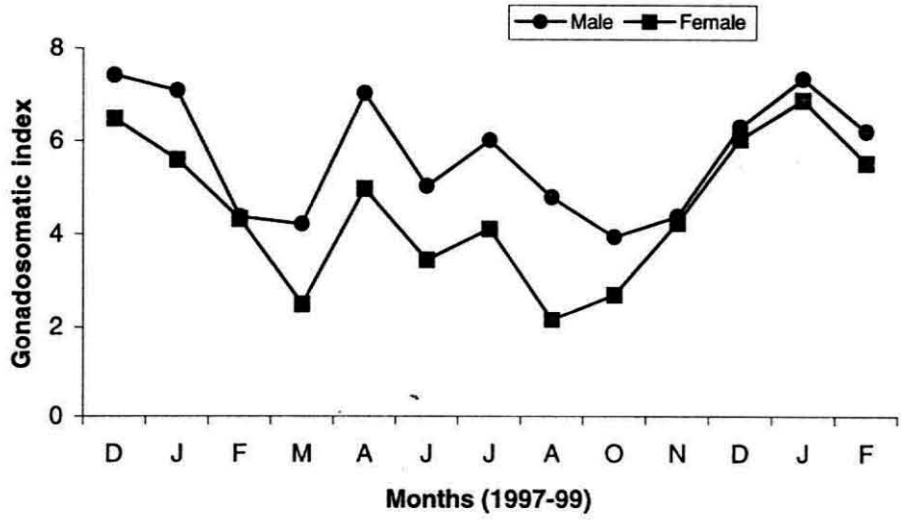


Fig.4. Monthly variations in mean gonadosomatic indices of male and female *H. varia* at Mandapam station during the study period.

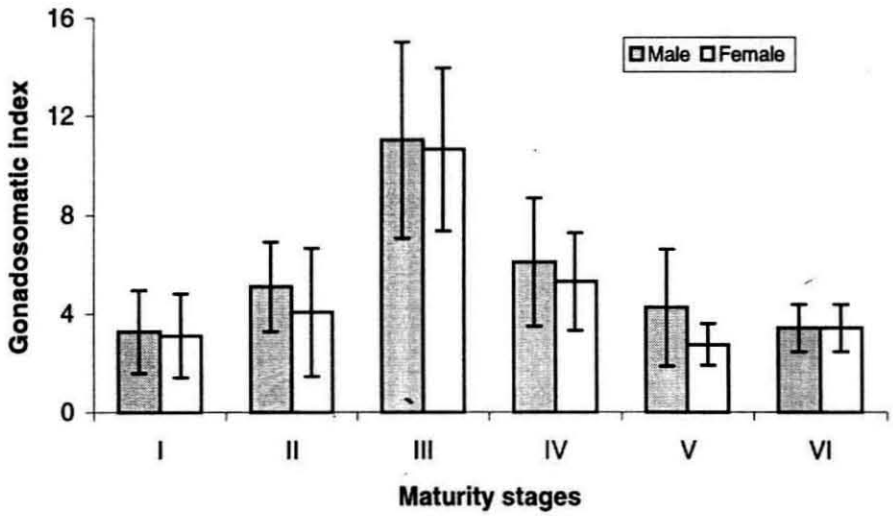


Fig. 5. Mean gonadosomatic indices at different maturity stages of male and female *H. varia* from Tuticorin station. Vertical bars represent standard deviations

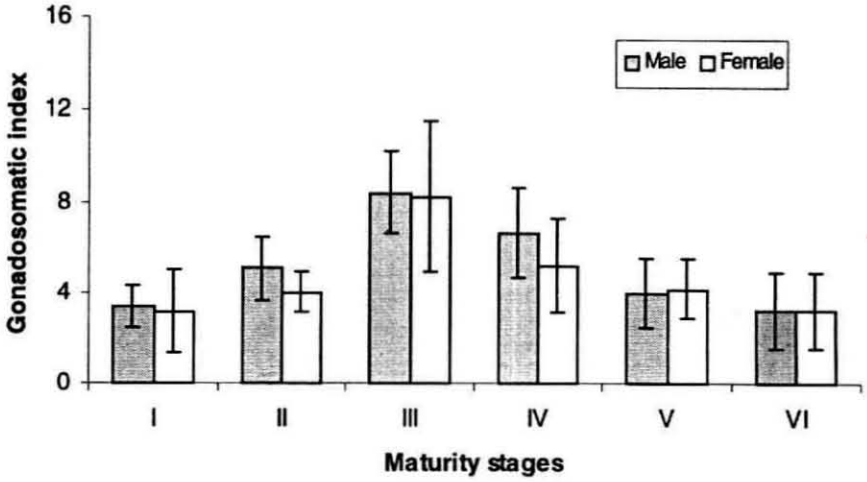


Fig. 6. Mean gonadosomatic indices at different maturity stages of male and female *H. varia* from Mandapam station. Vertical bars represent standard deviations

3.2. Sex ratio

In this study a total of 618 animals were examined from the Tuticorin station for their sex ratio calculation. Females slightly outnumbered the males but still they were found to be 1:1 ratio. The total sex ratio in Tuticorin was 274 males: 300 females and 44 indeterminate animals.

From the Mandapam station, totally 466 animals were used for determining the sex ratio. Here, both the sexes appeared in equal numbers with 223 males and 221 females. Only 19 indeterminate animals were encountered during the study period. The total sex ratio and month-wise sex ratio from Tuticorin and Mandapam stations are shown in tables 7 and 8.

Chi-square tests were performed against a 1:1 ratio. The values were nonsignificant and the males and females were found to be in 1:1 ratio in both the stations.

3.3. Size at first sexual maturity

Some idea of sexual maturity may be obtained by noting the size at which gametes were first produced. The results of such observations from both Tuticorin and Mandapam stations are shown in tables 9 and 10. The male and female abalones were grouped into 2 mm class intervals for this purpose. As males and females developed their first gametes at different sizes, the sexes have been separately given in the table. On plotting the data, it is evident that in Tuticorin population, 50% of the males can be expected to have gametes at a shell length of 18 to 20 mm. Among females, 50% of maturity obtained was at shell length of 22 to 24 mm. At a shell length of 28 to 30 mm all members of the population have gametes.

Table 7. Monthly distribution of sex ratio in adult *H. varia* at Tuticorin during the study period.

Month	Male	Female	Chi-square value
December	16	14	0.133
January	10	19	2.793
February	18	9	3.000
March	16	14	0.133
April	22	12	2.941
May	13	14	0.037
June	13	16	0.360
July	10	18	2.286
August	12	16	0.429
September	30	17	3.596
October	24	34	1.724
November	26	39	2.600
December	34	30	0.250
January	13	25	3.789
February	17	23	1.500
Total	274	300	1.143

Table 8. Monthly distribution of sex ratio in adult *H. varia* at Mandapam during the study period.

Month	Male	Female	Chi-square value
December	19	20	0.026
January	19	8	4.481
February	13	14	0.037
March	15	11	0.615
April	16	15	0.032
June	16	12	0.571
July	15	19	0.471
August	12	15	0.333
October	22	28	0.720
November	22	30	1.231
December	17	17	0.000
January	19	16	0.257
February	18	16	0.118
Total	223	221	0.009

Table 9. Size at first sexual maturity of *H. varia* at Tuticorin station

Shell length (mm)	Percentage mature	
	Male	Female
12.0-14.0	0.0	0.0
14.0-16.0	0.0	0.0
16.0-18.0	0.0	0.0
18.0-20.0	75.0	25.0
20.0-22.0	80.0	33.3
22.0-24.0	92.1	54.5
24.0-26.0	94.3	66.7
26.0-28.0	100.0	78.6
28.0-30.0	100.0	100.0

Table 10. Size at first sexual maturity of *H. varia* in Mandapam station

Shell length (mm)	Percentage mature	
	Male	Female
12.0-14.0	0.0	0.0
14.0-16.0	0.0	0.0
16.0-18.0	0.0	0.0
18.0-20.0	20.0	0.0
20.0-22.0	50.0	0.0
22.0-24.0	80.0	50.0
24.0-26.0	83.3	62.5
26.0-28.0	95.5	87.5
28.0-30.0	100.0	100.0

For the Mandapm population, the onset of sexual maturity among males was observed at a shell length of 20 to 22 mm. But that for females was at the shell length of 22 to 24 mm. In the Tuticorin population, the smallest female abalone with apparent mature eggs was at a shell length of 19.22 mm; whereas that for Mandapam was little larger with a shell length of 23.44 mm.

3.4. Fecundity

In general, the fecundity in the tropical abalone species is lesser than that in the temperate abalones. The number of eggs calculated in individual *H. varia* ranged from 15,160 in 26.66 mm to 2,75,663 at 48.04 mm. The relationship of fecundity with shell length (L), total weight (W) and gonad weight (G) of the abalone were also calculated. Shell length-fecundity plots showed a curvilinear relationship and plots of total weight and gonad weight versus fecundity appeared linearly related. Fecundity, shell length, total weight and gonad weight were transformed to logarithms (base 10) and by least square method the following equations were obtained.

$$\text{Log } F = -1.8785 + 4.2866 \text{ Log } L ; \qquad r = 0.855$$

$$\text{Log } F = 3.5018 + 1.4957 \text{ Log } W ; \qquad r = 0.863$$

$$\text{Log } F = 5.3329 + 1.5614 \text{ Log } G ; \qquad r = 0.735$$

Where F = Fecundity, L = Shell length, W = Total weight and G = Gonad weight.

Correlation coefficient (r) was significant ($P < 0.01$) for all three relationships. Scatter diagrams and fitted lines are shown in Figure 13. Fecundity of *H. varia*, collected from Tuticorin station is given in the table 11.

3.5. Hepatosomatic Index

Generally, biochemical synthesis in gonads is preceded by storage of organic reserves in other tissues. Hence, sizes of the storage organ would increase prior to gonadal development, then decrease during gonadal development as nutrients are mobilized from storage organ to gonad (Casille and Lawrence, 1991). Digestive gland of *H. varia* lies over the gonad and forms a part of the conical appendage. The monthly changes in the hepatosomatic index (HSI) were calculated for both the populations. HSI for different stages was also determined for both the stations (Tables 1 and 2). In all the cases HSI was lowest in stage III when the GSI was the highest. The HSI was lower in the stages I and II in most of the cases. The mean HSI for male and female animals at different maturity stages in both the stations are given in Tables 1 and 2.

3.6. Progression of ova towards maturity

With a view to trace the development of ova from the early maturing stage to the ripe condition, oocyte size-frequency profiles were constructed at various developmental stages of *Haliotis varia* (Fig. 14). The ova diameter measurements were grouped into 10 μm intervals. Stage I had only immature oocytes ranging from 10 μm to 57 μm. A prominent mode was observed at 45 μm. In stage II, the gradual growth of oocytes was discernible with the largest oocyte diameter (LOD) of 148 μm, having a mode at 68 μm and with a diameter range of 45 – 148 μm. Stage III (ripe) ovary showed clear and well differentiated eggs with a mode at 171 μm and the LOD 228 μm. In stage IV, the oocyte diameter ranged from 137 μm to 228 μm, with the same mode as ripe ovary (171 μm).

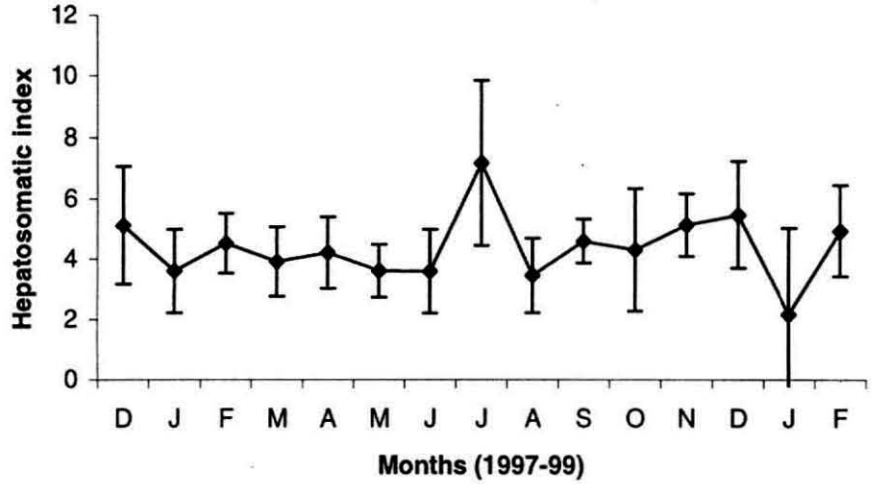


Fig. 7. Monthly variation in the hepatosomatic index of *H. varia* at Tuticorin station during the study period. Vertical bars represent standard deviations.

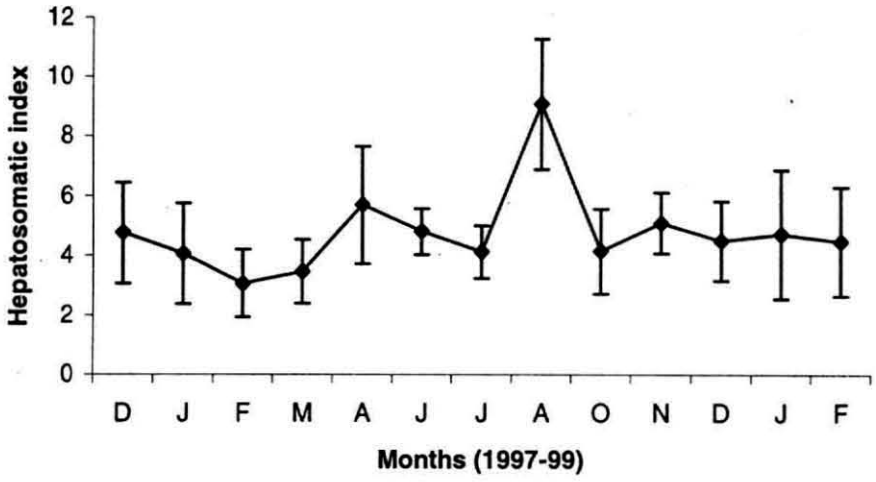


Fig. 8. Monthly variation in the hepatosomatic index of *H. varia* at Mandapam station during the study period. Vertical bars represent standard deviations.

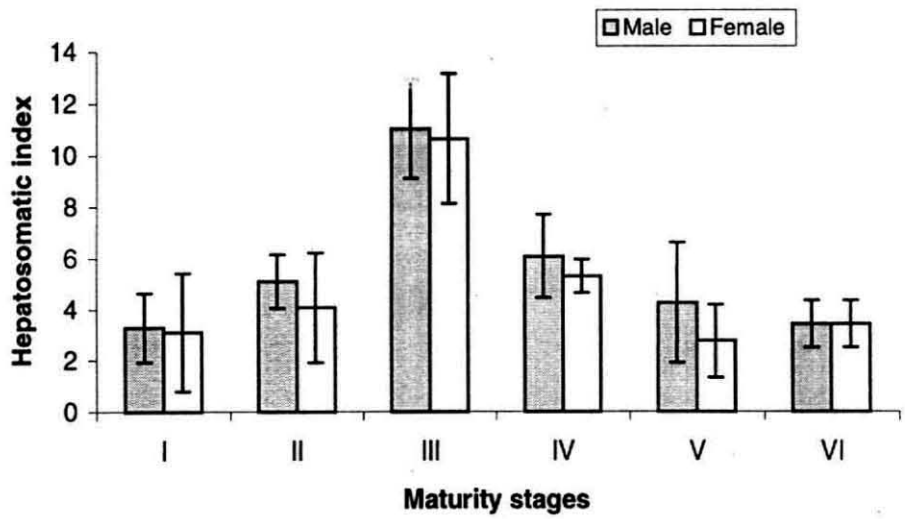


Fig. 9. Mean hepatosomatic indices at different maturity stages of male and female *H. varia* from Tuticorin station. Vertical bars represent standard deviations

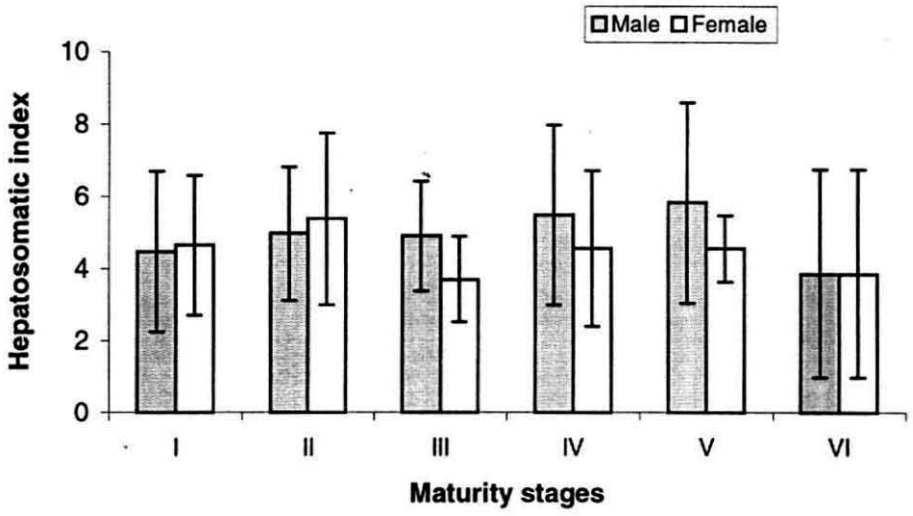


Fig.10. Mean hepatosomatic indices at different maturity stages of male and female *H. varia* from Mandapam station. Vertical bars represent standard deviations.

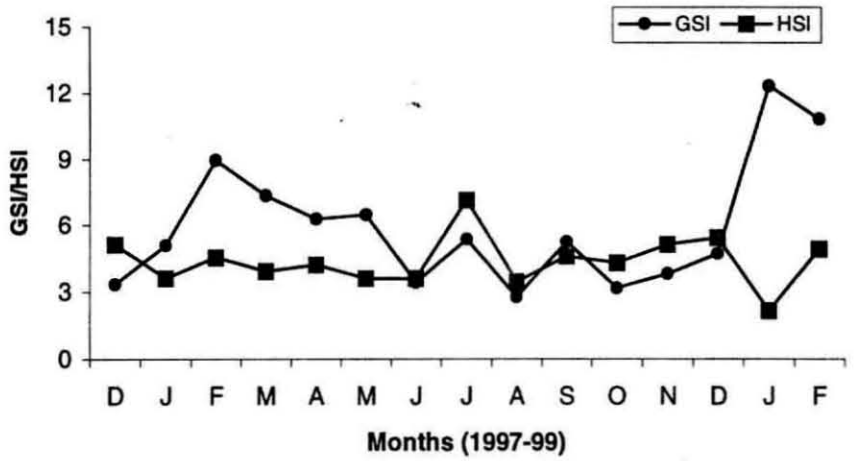


Fig. 11. Monthly vatiations in Gonadosomatic index and Hepatosomatic index of *H.varia* during the study period at Tuticorin station showing inverse relationship.

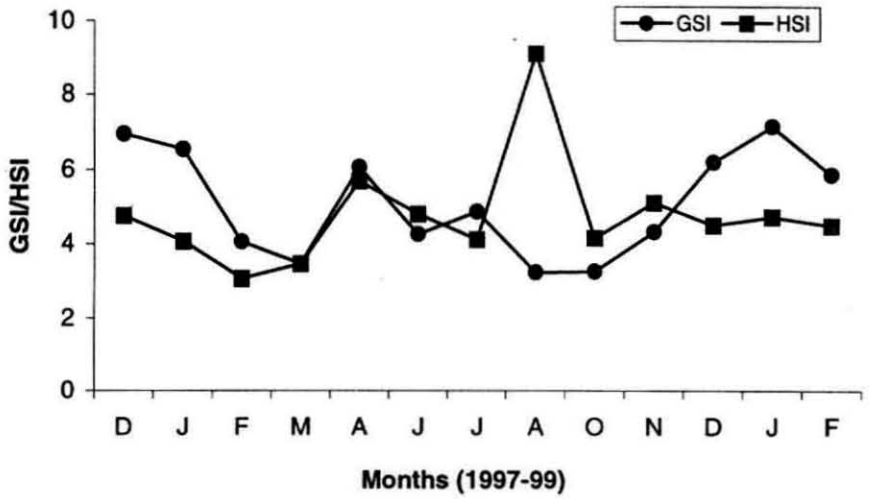


Fig. 12. Monthly vatiations in Gonadosomatic index and Hepatosomatic index of *H. varia* during the study period at Mandapam station showing inverse relationship.

Table 11. Fecundity of *H. varia* collected from the Tuticorin station.

No	Shell length (mm)	Total weight (gms)	Gonad weight (gms)	Fecundity
1	26.66	2.986	0.176	15,160
2	31.64	5.656	0.409	30,314
3	34.26	5.887	0.334	38,973
4	34.96	5.992	0.436	63,218
5	35.50	7.397	0.667	1,28,120
6	36.20	8.014	0.453	74,527
7	36.78	8.755	0.623	20,822
8	37.56	8.534	0.528	84,325
9	38.32	8.644	0.459	53,932
10	40.04	9.321	0.605	95,369
11	48.12	15.960	0.674	2,10,588
12	48.04	15.115	0.744	2,75,663
13	32.40	5.571	0.368	80,165

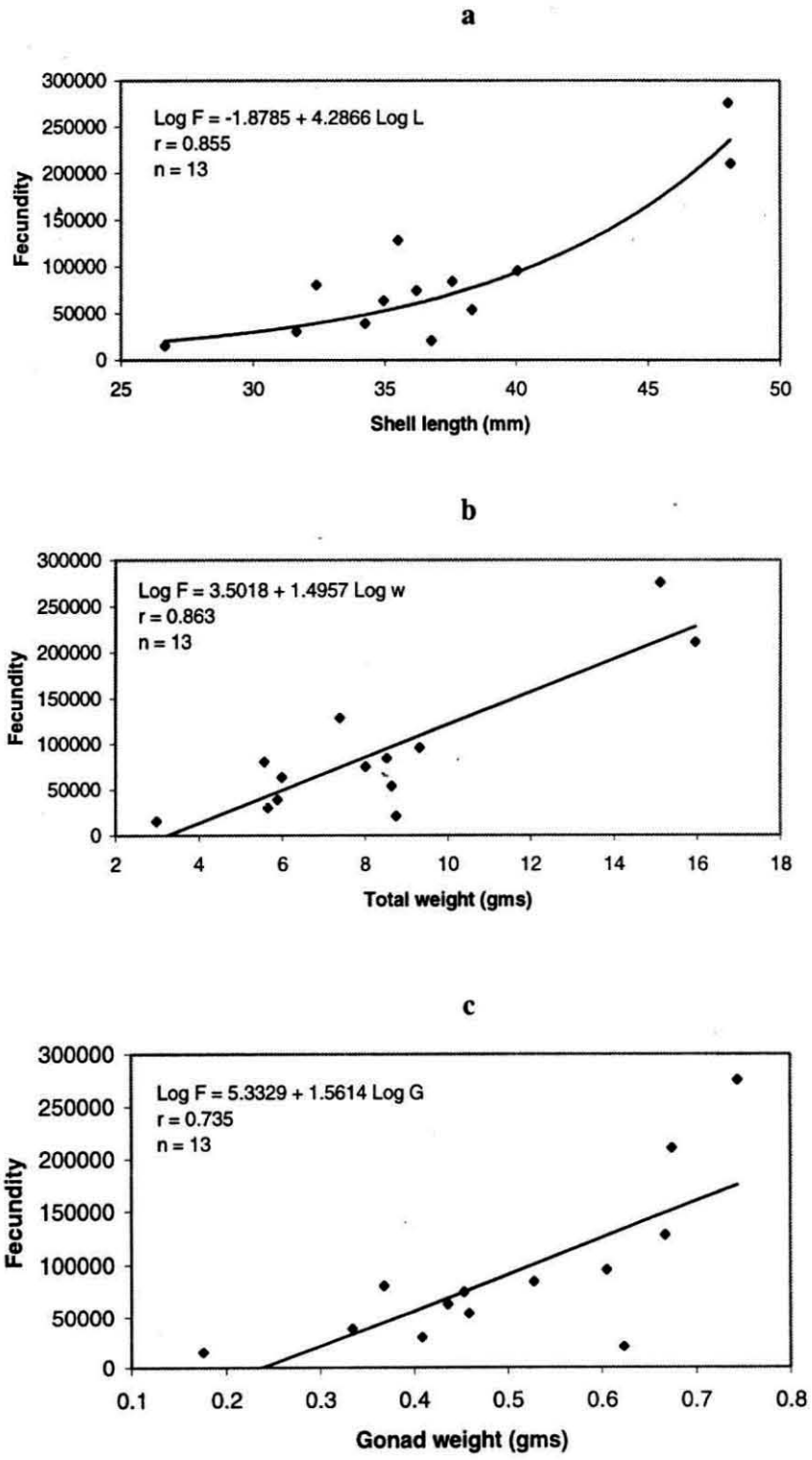


Fig. 13. Relationship between fecundity of *H. varia* and a) Shell length (L) b) Total weight (W) and c) Gonad weight (G).

Table 12. Oocyte diameter- frequency profiles at various maturity stages of *H. varia*

Oocyte diameter range (µm)	Percentage of oocytes			
	Stage I	Stage II	Stage III	Stage IV
11 – 20	1	-	-	-
21 – 30	14	-	-	-
31 – 40	32	2	-	-
41 – 50	37	12	-	-
51 – 60	14	16	-	-
61 – 70	1	29	-	-
71 – 80	-	11	-	-
81 – 90	-	0	-	-
91 – 100	-	8	-	-
101 – 110	-	8	-	-
111 – 120	-	5	-	-
121 – 130	-	3	-	-
131 – 140	-	4	4	4
141 – 150	-	3	2	16
151 – 160	-	-	13	15
161 – 170	-	-	5	0
171 – 180	-	-	31	17
181 – 190	-	-	25	12
191 – 200	-	-	11	15
201 – 210	-	-	4	8
211 – 220	-	-	2	7
221 – 230	-	-	3	6
Oocyte diameter mode	45 µm	68 µm	171 µm	171 µm
LOD (Largest Oocyte Diameter)	57 µm	148 µm	228 µm	228 µm

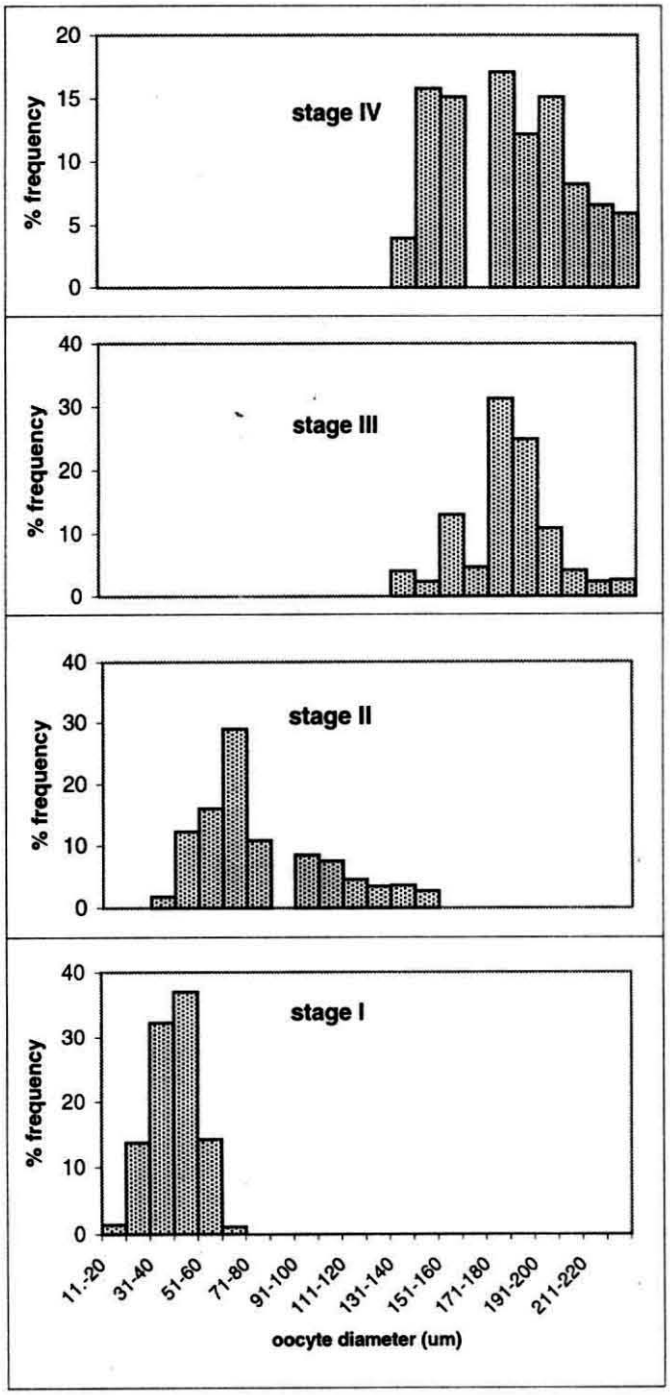


Fig. 14. Oocyte diameter-frequency profiles corresponding to stages of ovarian recrudescence in *Haliotis varia*

Almost all the oocytes when grown to a diameter of 57 μm entered the phase of vitellogenesis. Yolky oocytes at a size of 160 μm had a vitelline membrane around the oocyte membrane. The range of oocytes in the various developmental stages of *H. varia* is shown in the table 12.

4. HISTOLOGY OF THE GONAD

4.1. Mechanism of spermatogenesis

Spermatogenesis in *H. varia* takes place in the lumen of the testis. As evident in the light microscopic studies, the germinal epithelium covers the connective tissue tubes as well as the inner walls of the testis. Electron microscopic studies revealed that a series of steps were involved in the formation of mature spermatozoan from the germinal epithelium. The germinal epithelium in the lumen gave rise to spermatogonia. Spermatogenesis was observed to progress towards the central lumen and digestive gland area of the testis and therefore subsequent developmental stages like spermatocytes and spermatids were found in the central lumen of the testis. The spermatogonial cells were observed surrounding the connective tissue and the inner wall of the testis. Each spermatogonium passed through a period of quick growth to become a primary spermatocyte. The primary spermatocyte then divided mitotically to form two secondary spermatocytes. These divided again to produce four spermatids, which developed without further division to spermatozoa.

Since spermatogenesis involved progressive reduction of cytoplasm and condensation of cytoplasm to produce spermatozoa, the spermatogonial cells were observed as larger than spermatocytes, which in turn were larger than spermatids. As cytoplasmic limits of spermatogonial cells and spermatocytes were not clearly

apparent most of the cases, only nuclear diameter were used to classify the gonial cells.

Spermatogonia

The spermatogonia of *H. varia* were characterized by a more or less oval nucleus with a diameter of 5.33 μm. The nucleus contained small clumps of electron dense chromatin, which were often associated with inner nuclear membrane (Plate XI1). The cytoplasm of the spermatogonial cells was much smaller compared to the large nucleus. Several small mitochondria and small dense granular materials were distributed in the cytoplasm. There was no nucleolus evident in the nucleoplasm of the spermatogonial cells. The number of spermatogonial cells was more in the early maturing stage of the testis (Plate XI2). They were found to be distributed around the connective tissue tubules and along the inner walls of the testis.

Primary spermatocyte

Spermatogonial cells divided mitotically and developed in to primary spermatocytes, which were smaller. The primary spermatocytes of *H. varia* had a nucleus that was similar to that of spermatogonial cells. It measured about 4.23 μm in diameter. The chromatin was found to be more dispersed within the nucleus. The cytoplasm was a narrow rim around the large nucleus and contained several mitochondria, Golgi bodies, lipid inclusions and free ribosomes. The presence of dark round proacrosomal vesicles was evident in the cytoplasm of primary spermatocytes (Plate XI3). They were membrane bound dense osmeophilic granules, which were formed by Golgi bodies and responsible for the formation of acrosome of the spermatozoa.

Secondary spermatocyte

The secondary spermatocyte was formed by the first maturation division of primary spermatocyte. The secondary spermatocytes of *H. varia* were dominated by a spherical nucleus with a diameter of 4 μm (Plate XI4). The nucleus was little more condensed than the primary spermatocytes with more dense chromatin materials. A few mitochondria, ER and small vacuoles were present in the cytoplasm. More number of proacrosomal vesicles was evident in the cytoplasm than that in the primary spermatocytes.

The primary and secondary spermatocytes were abundant in the late maturing stage of the testis. They were found to be arranged little bit away from the periphery of the connective tissue tubules.

Spermatids

The secondary spermatocytes developed in to spermatids by way of the second maturation division. The spermatids of *H. varia* can be distinguished into early spermatid, mid spermatid and late spermatid stages, depending on the size and nature of the cytoplasm and nucleus. The nucleus of the early spermatid was more or less ellipsoidal with a diameter of 3.43 μm (Plate XI5). The nuclus was more condensed than both the spermatocytes and contained a network of electron dense and lucent patches of fine granular heterochromatin. The cytoplasm contained numerous mitochondria, Golgi bodies, centreoles and more number of proacrosomal vesicles.

In the mid spermatid, the nucleus was irregularly shaped and condensed into an electron dense body with a diameter of 2.06 μm (Plate XI6). The proacrosomal

vesicles increased in number and size. Some mitochondria and Golgi bodies were evident in the cytoplasm.

As the spermatid matured, the cytoplasm was lost by sloughing. The nucleus of late spermatid was much smaller with a diameter of 1.23 to 1.4 µm (Plate XII2). The nucleus became elongated and the nuclear contents continued to condense. The proacrosomal vesicles migrated to the presumptive anterior end of the spermatid, where they coalesced to form a single, electron dense vesicle, the acrosome. The mitochondria became reduced in number but increased in size. The larger mitochondria formed a close association with the nucleus.

As the further development of the spermatid occured, the mitochondria formed by the fusion of small mitochondria, came to occupy the end of the cell opposite the acrosome, thus forming the sperm midpiece. The mitochondria then measured 0.5 µm in diameter (Plate XII4).

The major developments in spermiogenesis was the continued loss of cytoplasm, decrease in nuclear size and condensation of nuclear material of spermatids. This stage was characterized by further development of the acrosome. The spherical acrosome became oval with the short axis in the anterior posterior plane of the spermatid. The acrosome then began to migrate on its ad nuclear surface and elongate, forming the characteristic conical shaped acrosome.

Structure of mature spermatozoan

Each mature spermatozoa of *H. varia* consisted of an anterior conical acrosome and nucleus (together constitute the head piece), a mid piece and a

flagellum (Plate XII6). The spermatozoa without the flagellum measured approximately 2.8 μm.

Acrosome

The acrosomal vesicle of *H. varia* spermatozoa was conical, membrane bound and moderately electron dense. It measured 0.57 μm in length and 0.73 μm width at the base. Acrosome rested on a shallow conical invagination at the anterior end of the nucleus. A narrow deep invagination partially filled with a fibrous subacrosomal material and an axial rod extended from the base of vesicle anteriorly for a distance of 0.23 μm (Plate XII4). Anteriorly a spherical zone can be differentiated from the remainder of the acrosome contents by its slightly more intense electron density. No further differentiation of the acrosomal contents has been observed.

Observations on the developing primary spermatocyte during the early stages of spermatogenesis indicated the presence of small multiple proacrosomal vesicles throughout the cytoplasm. The vesicle contents were moderately electron dense, round and fine granular. In the early and mid spermatids, the definitive acrosomal vesicle was large and ovate or round with 0.22 μm diameter, derived presumably through the fusion smaller proacrosomal vesicles. As development proceeded these vesicles migrated to the presumptive anterior end of the spermatids, where they coalesced to form a single electron dense vesicle, which was the acrosome of the mature spermatozoa.

Nucleus

The nucleus of spermatozoa was elongate and barrel shaped with a length of 1.16 μm and 1.04 μm in width. Its contents were found to be intensely electron dense with exception of some irregularly shaped electron lucent lacunae. The apex of the

PLATE XI

1. Electron micrograph of a spermatogonial cell with oval nucleus (N- Nucleus, CH- Chromatin, NM- Nuclear membrane) X15000.

2. Light micrograph of a late maturing testis showing the arrangement of spermatogonial cells and spermatocytes (TB- Tubule, SG- Spermatogonaia, SC- Spermatocytes). X200.

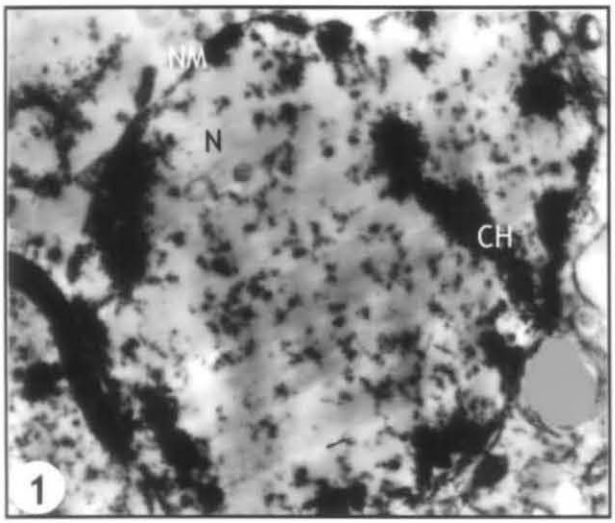
3. Electron micrograph of a primary spermatocyte with dispersed chromatin material (C- Cytoplasm, N- Nucleus, M- Mitochondria, CH- Chromatin, PAV- Proacrosomal vesicles, LI- Lipid inclusions). X17000.

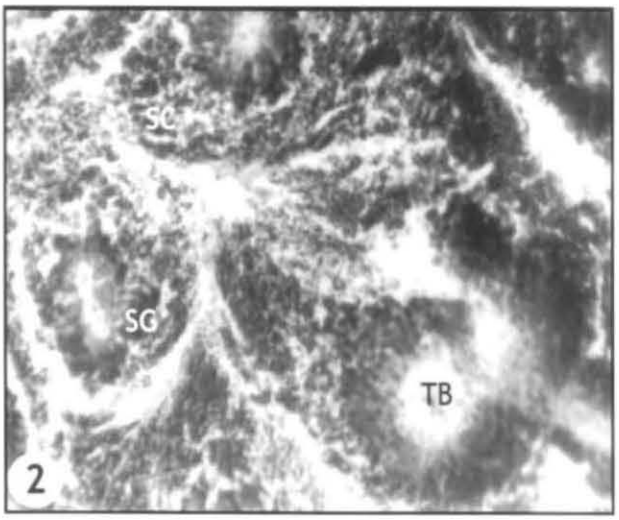
4. Electron micrograph of a secondary spermatocyte (N- Nucleus, CH- Chromatin, PAV- Proacrosomal vesicles). X12000.

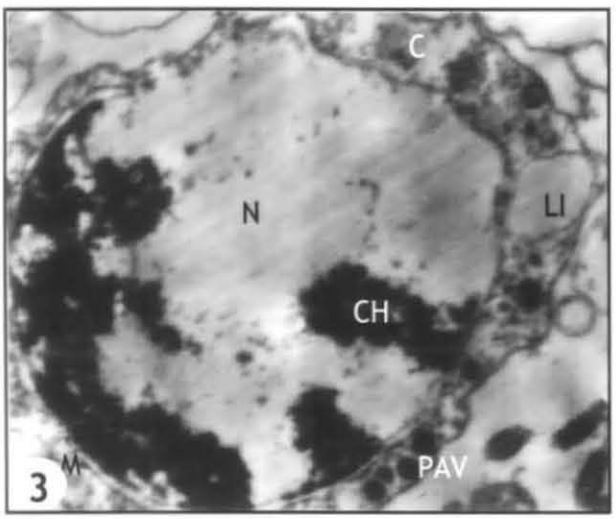
5. Electron micrograph of an early spermatid with ellipsoidal nucleus (C- Cytoplasm, N- Nucleus, CH- Chromatin, R- Ribosomes, PAV- Proacrosomal vesicles). X17000.

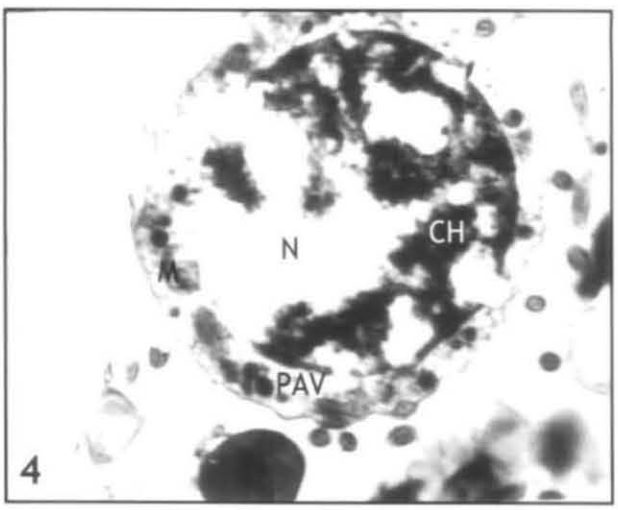
6. Electron micrograph of a midspermatid with highly condensed nucleus (C- Cytoplasm, N- Nucleus, M- Mitochondria, R- Ribosomes, L- Lipid inclusions, PAV- Proacrosomal vesicles). X17000.

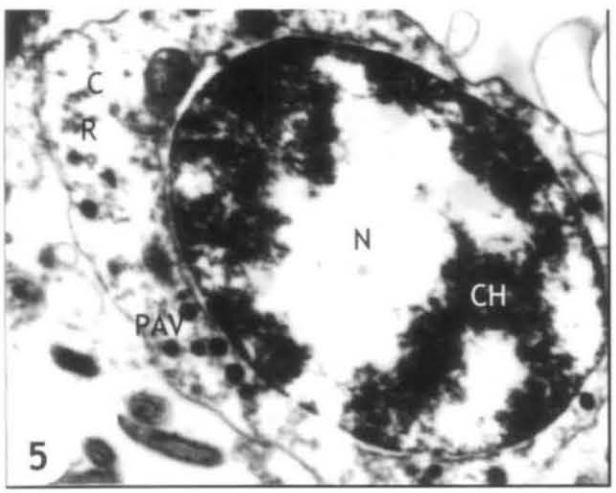
PLATE XI


NM
N
CH
1


SG
SG
TB
2


C
N
LI
CH
M
PAV
3


N
CH
M
PAV
4


C
R
N
CH
PAV
5

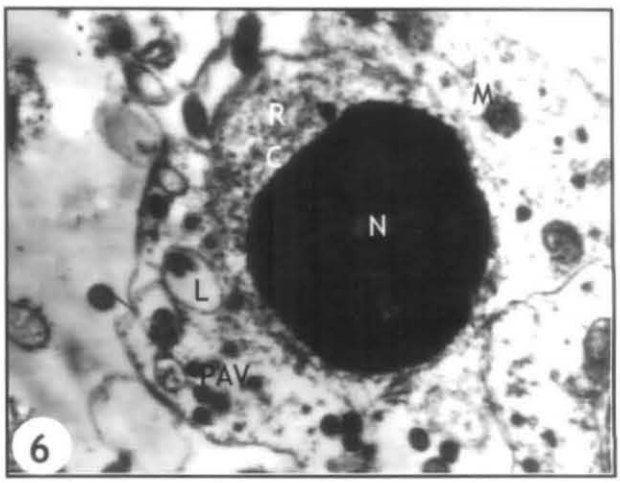

M
R
C
N
L
PAV
6

PLATE XII

1. Electron micrograph of section through the testis showing early spermatids and midspermatids (ES- Early spermatid, MS- Midspermatid, LS- Late spermatid, TL- Testicular lumen, M- Mitochondria, PAV- Proacrosomal vesicles). X8000.

2. Electron micrograph of a late spermatid (C- Cytoplasm, N- Nucleus, AV- Acrosomal vesicle). X3000.

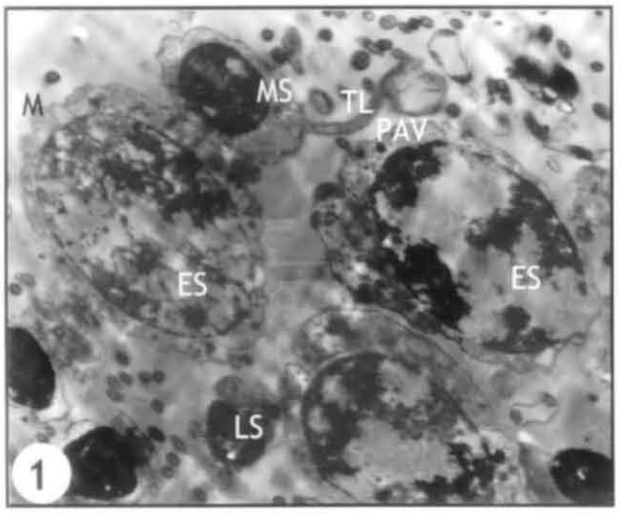
3. Low power electron micrograph of the lumen of the late maturing testis with mid and late spermatids (TL- Testis lumen, MS- Mid spermatid, LS- Late spermatid). X5000.

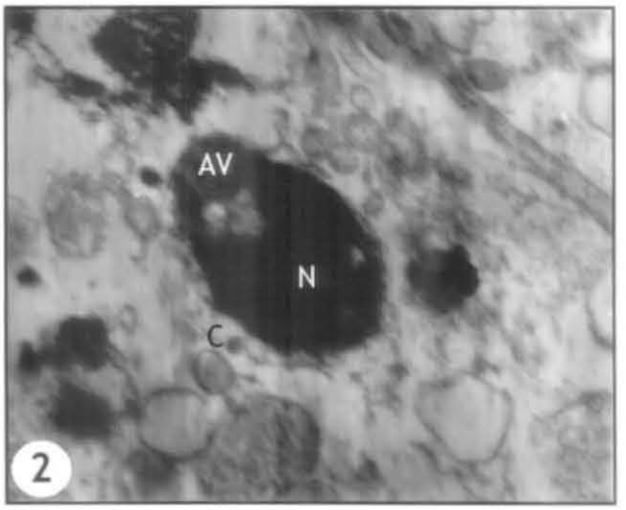
4. Electron micrograph of the mature spermatozoon showing the nuclear acrosomal structures (N- Nucleus, AV- Acrosomal vesicle, AF- Acrosomal fossa, M- Mitochondria, PAV- Proacrosomal vesicle). X3000.

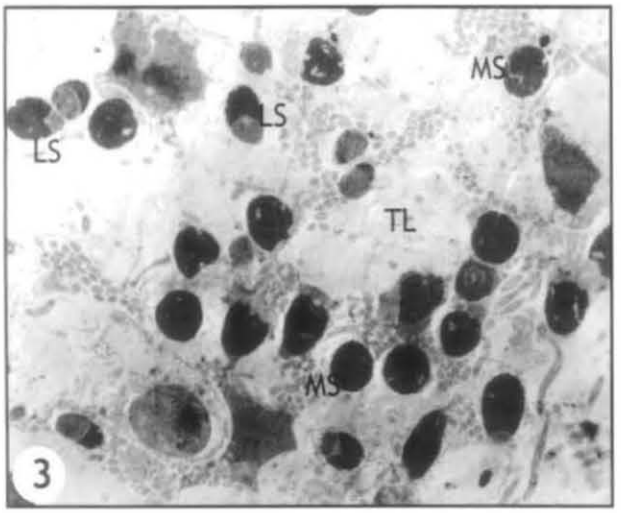
5. Section through the midpiece of the spermatozoon showing the arrangement of mitochondria (M- Mitochondria). X3000.

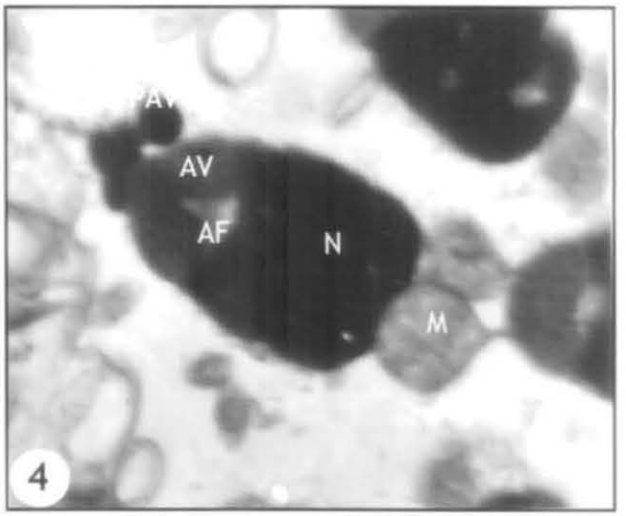
6. Transmission electron micrograph of a mature spermatozoon with details (N- Nucleus, M- Mitochondria, AC- Acrosome, D- Nuclear depression, T- Tail). X25000.

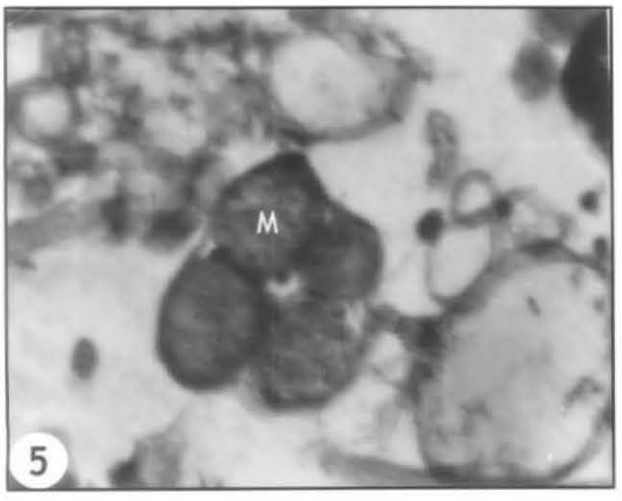
PLATE XII











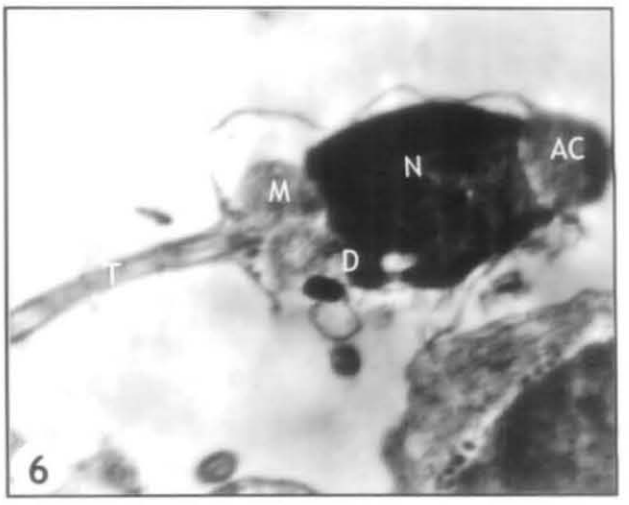


PLATE XIII

1. Electron micrograph of mature spermatozoon and early spermatozoon (M- Mitochondria, N- Nucleus, SP- Spermatozoon, ESP- Early spermatozoon, T- Tail). X2000.

2. Ultrastructural details of a mature spermatozoon (N- Nucleus, LC- Lacunae, W- Nuclear wall, M- Mitochondria, CE- Centriole, T- Tail). X35000.

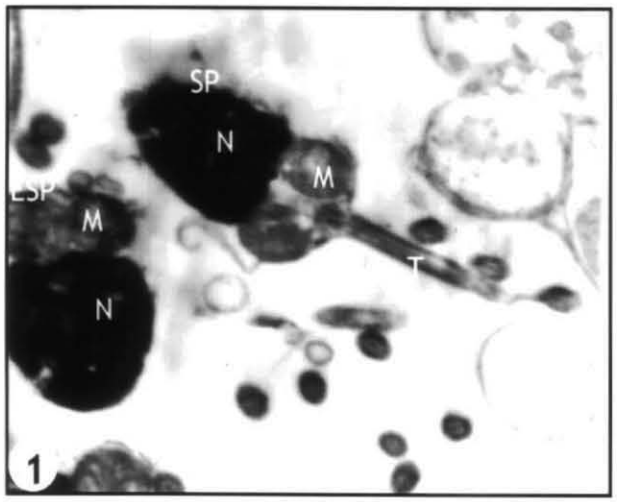
3. Section through the lumen of early maturing testis showing germinal cells and trabeculae (OL- Ovarian lumen, GC- Germinal cell, TB- Trabeculae, N- Nucleus, NL- Nucleolus, L- Lipid inclusions, G- Golgi complex). X6000.

4. Electron micrograph of an oogonium (OG- Oogonium, C- Cytoplasm, N- Nucleus, M- Mitochondria, R- Ribosomes, CH- Chromatin, GC- Germinal cells). X5000.

5. Ultrastructural details of the oogonium showing nuclear emissions (C- Cytoplasm, N- Nucleus, GNC- Granular nuclear emissions, R- Ribosomes, M- Mitochondria, CH- Chromatin, GC- Germinal cell). X10000.

6. Low power electron micrograph of a primary oocyte (C- Cytoplasm, N- Nucleus, NL- Nucleolus, CH- Chromatin, L- Lipid inclusions) X4000.

PLATE XIII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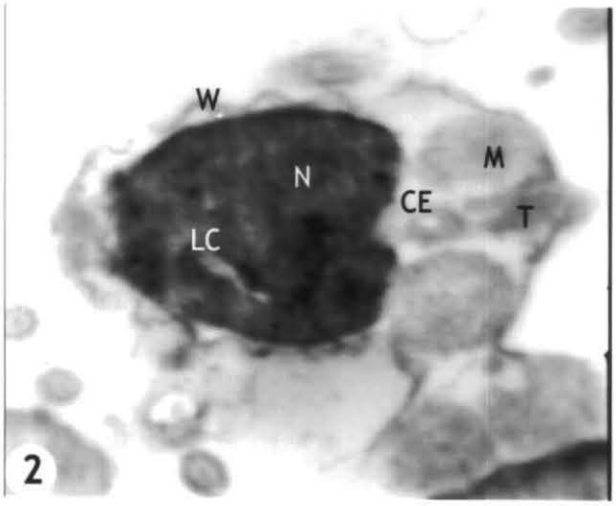
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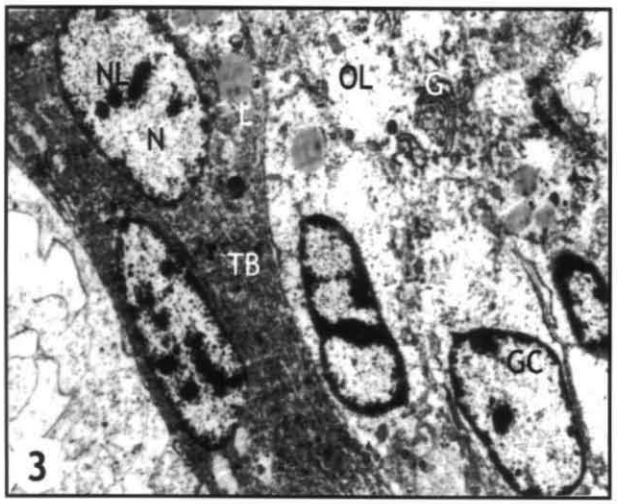
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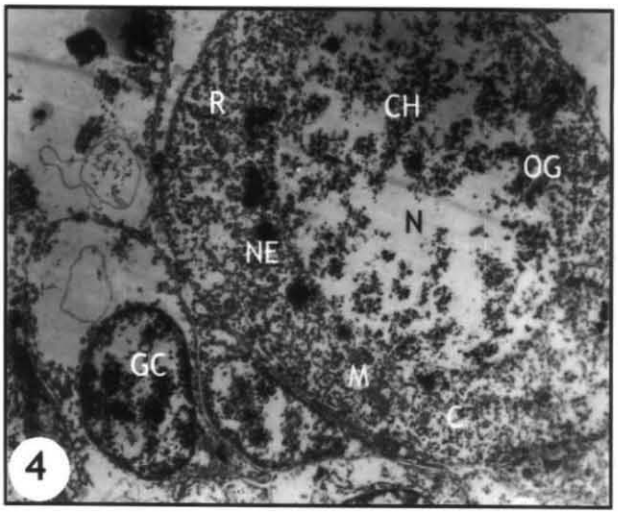
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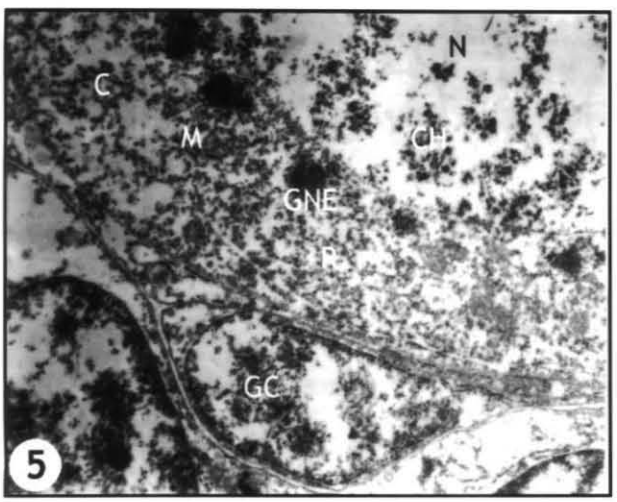
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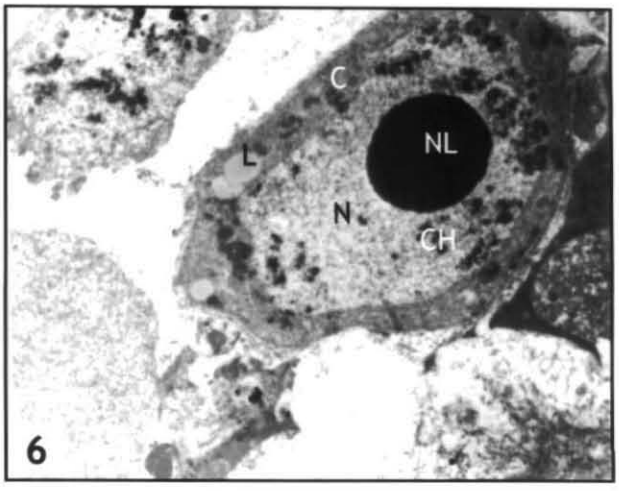
C
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L
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6

nucleus was slightly depressed, with an anterior 0.13 μm deep fossa filled by a flocculent subacrosomal material. Posteriorly, the nucleus has shallow depressions into which the mitochondria join and a small centrally located fossa housing the proximal centriole. The sperm nucleus was enclosed by its own double membrane apart from the loose sperm plasma membrane.

Mid piece

The mid piece was formed by four rounded mitochondria (Plate XII5), which were grouped around the proximal and distal centrioles (Plate XI2). Each mitochondrion has a diameter of 0.5 μm and internally contains several flattened cristae arranged in semi radial pattern. The centrioles were cylindrical with a diameter of 0.2 μm. The proximal centriole was attached to the basal fossa of the nucleus by a dense deposit. The distal centriole formed the basal body of the flagellum. The centriole was formed by nine triplets of satellite fibres.

Flagellum

Flagellum formed the tailpiece of the abalone spermatozoa. The flagellum was composed of the typical 9+2 axoneme and slender and long. The exact length of the flagellum was not measured. The measurement obtained in the ultra sections was 1.35 to 1.8 μm. But it may exceed more than that, because during sectioning the posterior region of the flagellum might be cut off from the spermatozoa.

4.2. Mechanism of oogenesis

Electron microscopic study of abalone ovary revealed that distinct steps were involved in the oogenesis of *H. varia*. Oogenesis involved two distinct process;

proliferative and differentiative. During the proliferative process, in the germinal zone the number of oogonial cells increased by mitotic multiplication. The primary oocytes, derived from the secondary oogonial cells were transformed into typical egg cells by differentiative process. In *H. varia* also the process of oogenesis passed through these stages. The proliferative phase of oogenesis was observed in early maturing and late maturing ovaries. The primordial germ cells, which line the inner ovarian wall, became primary oogonia. They then increased in number by mitotic division, thus forming secondary oogonia, which entered into the first mitotic division and became primary oocytes. During the differentiate phase it was observed that the oocytes accumulate their reserve yolk by the process called vitellogenesis and finally developed into mature oocytes.

Number of nuclear as well as cytoplasmic changes involved during the progress of oogenesis. The major changes included premeiotic phenomena in the nucleus and formation of germinal vesicle, synthesis of RNA in the germinal vesicle and storage in the cytoplasm, formation of variable numbers of yolk granules (vitellogenesis) and finally the oocyte maturation. Based on the changes in the size as well as cytoplasmic contents in the oocytes, five stages of oocytes can be distinguished in *H. varia*, which are dealt in detail.

Oogonia

In the ovary, the germinal epithelium lines the inside of the ovary. The primary oogonia were generally cuboidal and as they enlarge, they became oval or round secondary oogonia. The germinal cells of the ovary measured around 6 µm in diameter and the cuboidal nucleus measured 5 µm in length (Plate XIII3). The

oogonia had a diameter of 10 μm. They were dominated by a large ovoid nucleus with a diameter of 9 μm (Plate XIII4). The cytoplasm of oogonial cells contained numerous free ribosomes, mitochondria and some clusters of electron dense granules. One or two small germinal cells with large nucleus of diameter 5 μm can be seen attached on the base of each oogonial cell (Plate XIII5). The nucleus of secondary oogonial cells contained less condensed chromatin materials scattered in the nucleoplasm. Nucleolus was not conspicuous at any stage of oogonial cells. The oolemma was smooth and without any particular morphological specialization at this stage. The secondary oogonia entered into the first meiotic division and became primary oocytes.

Previtellogenic oocytes

The primary oocytes of *H. varia* measured 16-to18 μm in diameter and they became more elongated (Plate XIII6). Nuclear volume increased and it measured 12 μm in diameter. As the primary oocytes enlarged in size, they moved away from the germinal epithelium and toward the digestive gland, but remained attached by stalk and appear teardrop shaped. The cytoplasm of oocytes became strongly basophilic indicating considerable protein synthesis. As the enlargement of oocyte proceeded the nuclear cytoplasmic ratio of the oocyte decreased. The nucleoplasm contained less dense chromatin materials. A prominent electron dense nucleolus was discernible in the nucleus of the developing oocyte (Plate XIV1). The nucleolus was migrated to the distal portion of the nucleus. A clear electron loose round space was seen inside the dark electron dense nucleolus (Plate XIV2). Numerous gaps or nuclear pores were evident on the nuclear wall through which the electron dense nucleolar material was observed to diffuse into the cytoplasm (Plate XIV3). The cytoplasm of developing

oocyte contained numerous ribosomes, mitochondria, Golgi bodies as well as rough endoplasmic reticulam indicating active synthesis of protein. The secondary oocytes measured around 45 μm in diameter. A single layer of thin oolemma around the granular cytoplasm covered the oocyte at this stage.

Early vitellogenic oocytes

At about 50 μm in diameter, small, lightly stained spherical bodies started appearing in the cytoplasm of the developing oocytes (Plate XIV4), even in the places devoid of cell organelles. This was the onset of yolk synthesis ie., vitellogenesis. Lipid yolk in the form of round lipid droplets appeared in the cytoplasm of early vitellogenic oocyte. Later, protein yolk as darkly stained granules with a limiting membrane can be discernible along with the lipid droplets (Plate XIV5). Several cell organelles were seen in the cytoplasm of the oocyte, which actively participate in yolk formation. In *H. varia*, the vitellogenesis was found to be endogenous, ie., yolk formation was inside the oocyte and there was no extra cellular yolk material incorporated into the oocyte. No pinocitosis appeared in the oocyte, which indicated the endogenous nature of vitellogenesis.

Concentric layers of Rough endoplasmic reticulam were seen surrounding the lipid droplet (Plate XIV6) indicating their role in lipid yolk synthesis. Numerous mitochondria, free ribosomes and ribosomes attached to endoplasmic reticulam also were present in the cytoplasm. Concentric and closed layers of smooth endoplasmic reticulum encompassing several granules and vacuoles were discernible in the vicinity of the nucleus of early vitellogenic oocyte. These were the membrane specializations, which might be associated with the synthesis of yolk materials (Plate XV2). As

vitellogenesis started, they developed considerably often encompassing several granules and yolk materials. The exact nature of the materials encircled by these membrane specializations was not known. Other lamellar structures observed during vitellogenesis were annualate lamellae, which also originated in close proximity to the nucleus (Plate XV1). Large numbers of ribosome studded, as well as smooth membraned vesicles were also often present during the early stages of vitellogenesis.

Late vitellogenic oocytes

The cytoplasm of late vitellogenic oocyte was filled with various quantities of yolk granules and lipid droplets (Plate XV5). No cytoplasmic organelles were discernible at this stage except some mitochondria. Small granules also were seen in the cytoplasm. The nucleus was very large and migrated to the distal portion of the oocyte. Nucleus was less electron dense. A dark round electron dense nucleolus was evident in the nucleoplasm. The lipid droplets in the cytoplasm measured a maximum of 2.3 μm in diameter and yolk granules measured a maximum of 2.5 μm in diameter. A vitelline membrane was formed around the oolemma, which was around 2.3 μm in thickness . Numerous microvilli can be seen embedded in the vitelline membrane (Plate XV4). The microvilli were arranged 0.2 μm apart and their ends were slightly projecting out to the vitelline membrane. The abalone egg vitelline membrane was an amorphous thick layer, which covered the oolemma. The late vitellogenic oocyte measured 80 to 120 μm in diameter and seen attached to the free ends of the trabeculae in the ovarian lumen near the digestive gland. The number of late vitellogenic oocytes was more in the late maturing ovaries.

Mature oocytes

The fully mature oocyte of *H. varia* was having a well defined vitelline layer with microvillar projections. The nucleus was very large and less electron dense with a dark nucleolus. The cytoplasm was filled with lipid droplets and protein yolk granules. Cortical granules, the membrane bound vesicles, which release their contents extracellularly, were seen at the final stage of oogenesis in *H. varia* (Plate XVI1). They were round with a diameter of 0.3 μm and occupied as a layer beneath the cytoplasmic membrane of the mature oocyte. No cell organelles were discernible in the cytoplasm. Mature oocyte measured more than 140 μm in diameter and appeared as polymodal in shape. The oocytes were detached from the trabeculae and filled freely in the ovarian lumen. The ripe ovary of *H. varia* was filled with exclusively mature oocytes (Plate XV3).

Degenerating oocytes

Necrosis of vitellogenic oocytes was observed in all stages of the ovary. The first visible sign of necrosis occured in the nucleus. The chromatin became strongly oesnophilic and less granular. The nuclear membrane convoluted and broken down (Plate XVI2). The ooplasm became vacuolate and the perivitelline space between the oolemma and vitelline membrane increased. The microvilli were separated from the oocyte surface and finally the vitelline layer disintegrated (Plate XVI5). The yolk granules were covered with autophagic vacuoles (Plate XVI5). The rupture of the vitelline coat caused the release of vacuolated cytoplasmic contents to the ovarian lumen. Lysosomal vacuoles were often discernible in the lumen near the degenerating oocyte. Genital blood vessels also were evident near the degenerating

PLATE XIV

1. Electron micrograph of a primary oocyte (C- Cytoplasm, N- Nucleus, NL- Nucleolus, NE- Nuclear envelope, CH- Chromatin, L- Lipid inclusions). X5000.

2. Electron micrograph of the previtellogenic oocyte with nuclear details (C- Cytoplasm, N- Nucleus, NL- Nucleolus, ES- Electron loose space, NE- Nuclear emissions, R- Ribosomes, L- Lipid inclusions). X3500.

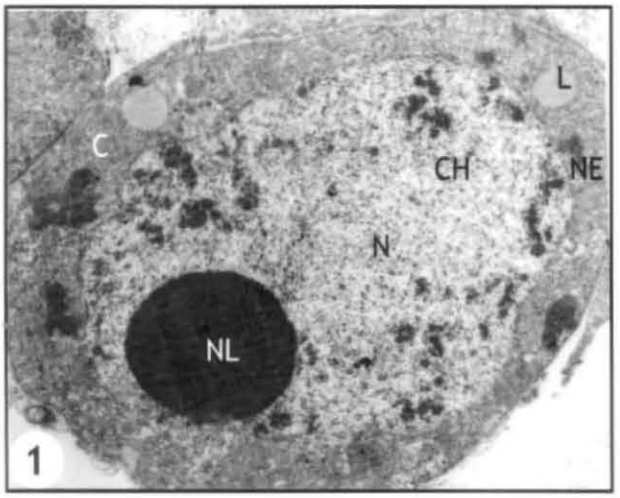
3. Ultrastructural details of the previtellogenic oocyte (C- Cytoplasm, N- Nucleus, Nucleolus, ES- Electron loose space, NM- Nuclear membrane, NP-Nuclear pores, NE- Nuclear emissions). X8000.

4. Electron micrograph of the cytoplasm of an earlyvitellogenic oocyte (C- Cytoplasm, LY- Lipid yolk, R- Ribosomes, M- Mitochondria, N- Nucleus, OM- Oocyte membrane). X5000.

5. Electron micrograph of early vitellogenic oocyte with yolk granules (PY- Protein yolk, LY- Lipid yolk, GR- Granules, OM- Oocyte, membrane). X8000.

6. Ultrastructure of membrane specializations composed of rough endoplasmic reticulum in the early vitellogenic oocyte (RER- Rough endoplasmic reticulm, LY- Lipid yolk, M- Mitochondria, R- Ribosomes). X20000.

PLATE XIV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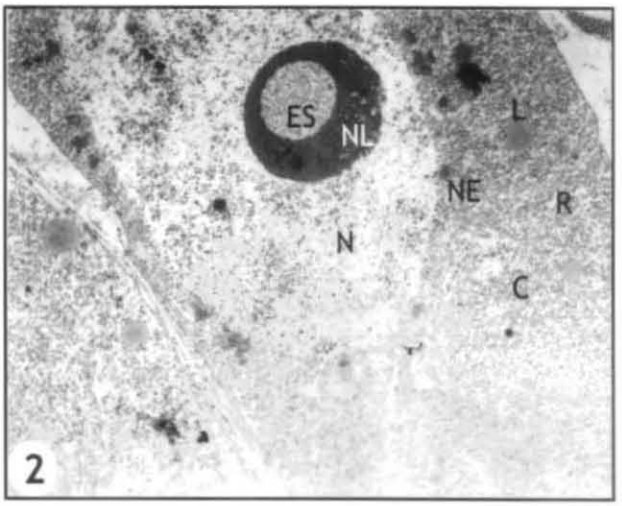
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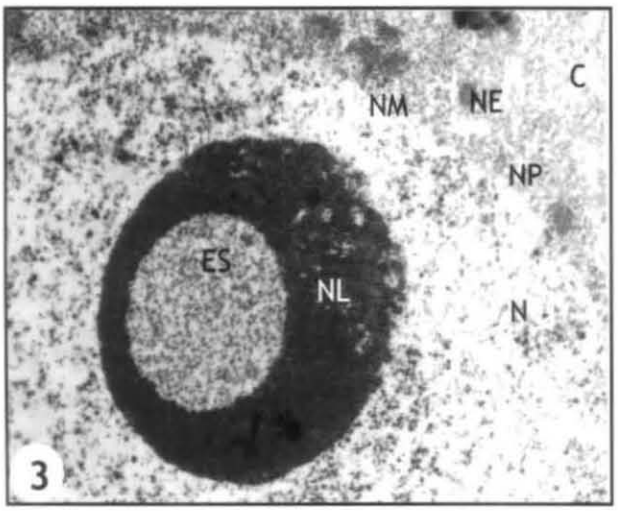
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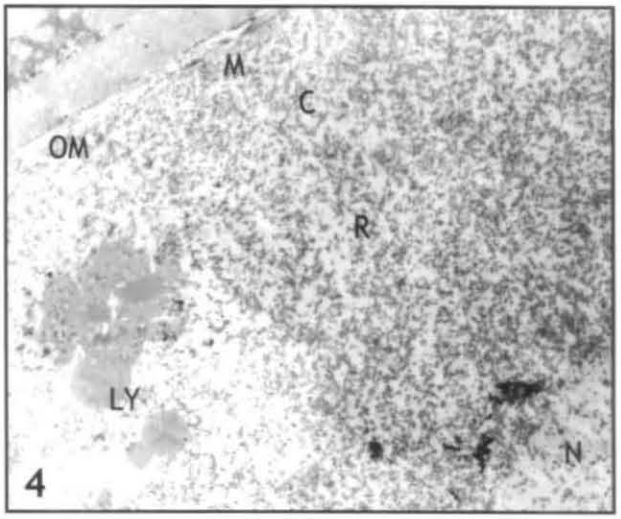
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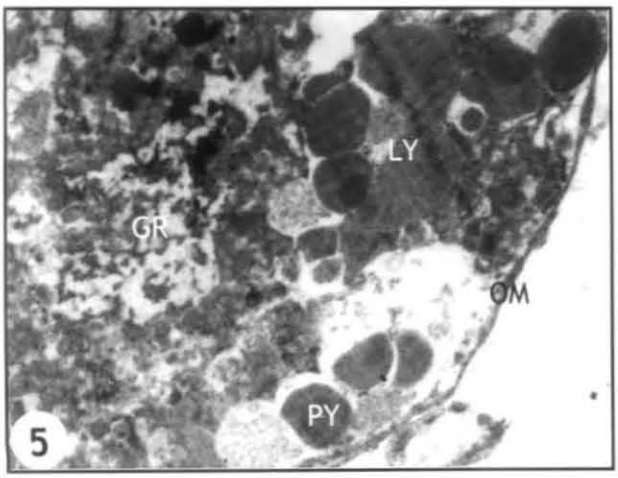
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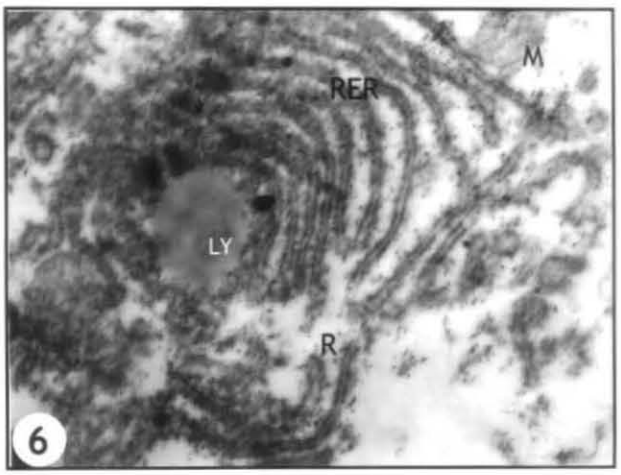

M
RER
LY
R
6

PLATE XV

1. Ultrastructure of the annulate lamellae associated with vitellogenesis (RER- Rough endoplasmic reticulum, PY- Protein yolk, LY- Lipid yolk). X12000.

2. Electron micrograph of membrane specializations composed of concentric layers of smooth endoplasmic reticulum in the early vitellogenic oocyte. (SER- Smooth endoplasmic reticulum, GR- Granules, V- Vacuoles). X20000.

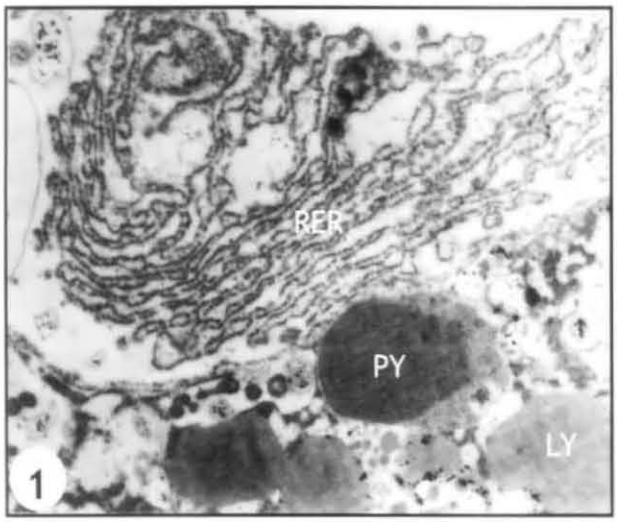
3. Light micrograph of a ripe ovary with tightly packed ripe oocytes (RO- Ripe oocyte, N- nucleus, Y- Yolk, NL- Nucleolus). X100.

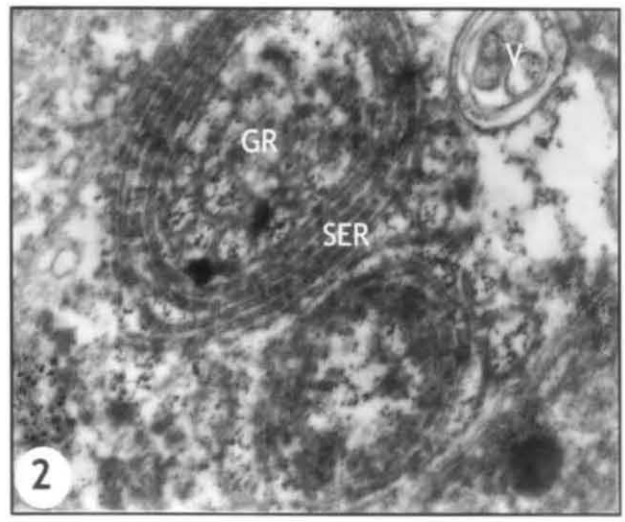
4. Electron micrograph of a mature oocyte showing the vitelline membrane with microvilli (VM- Vitelline membrane, MV- Microvilli, PM- Plasma membrane, CG- Cortical granules, LY- Lipid Yolk, PY- Protein yolk). X10000.

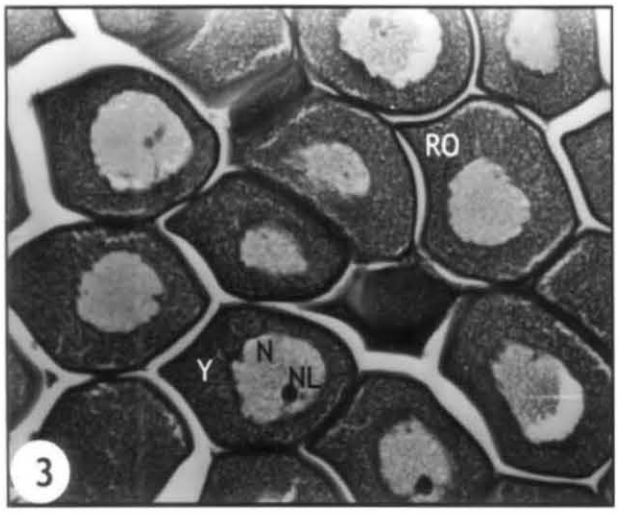
5. Ultrastructure of the cytoplasm of the mature oocyte with protein and lipid yolk (LY- Lipid yolk, PY- Protein yolk, M- Mitochondria, ER- Endoplasmic reticulum). X8000.

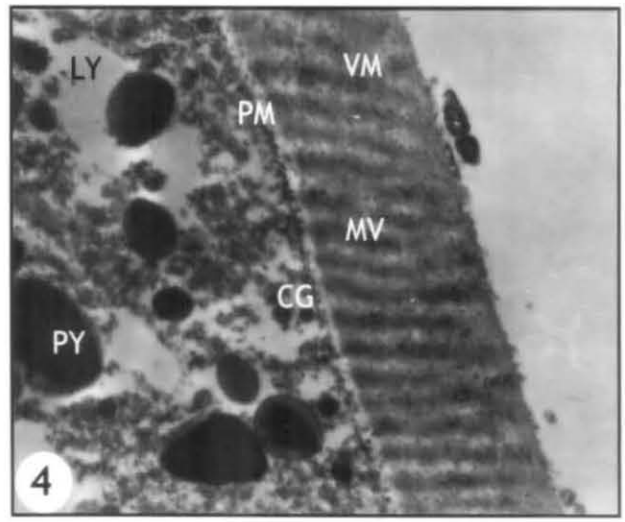
6. Ultrathin section through the microvilli in the vitelline membrane of the mature oocyte (MV- Microvilli). X15000.

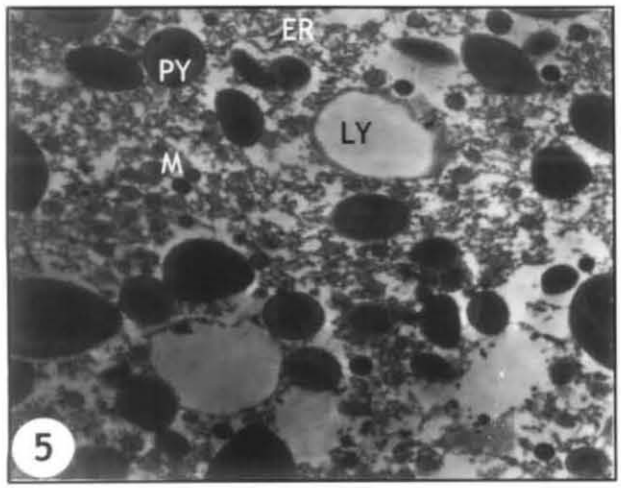
PLATE XV











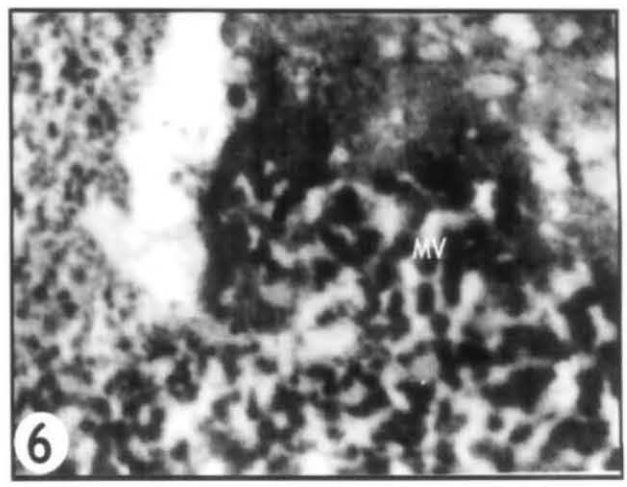


PLATE XVI

1. Electron micrograph of the periphery of the mature oocyte showing vitelline membrane and cortical granules (VM- Vitelline membrane, CG- Cortical granules, PM- Plasma membrane, LY- Lipid yolk). X12000.

2. Light micrograph of a degenerating oocyte in the partially spawned ovary (N- Nucleus, NMA- Nuclear material, VM- Vitelline membrane). X200.

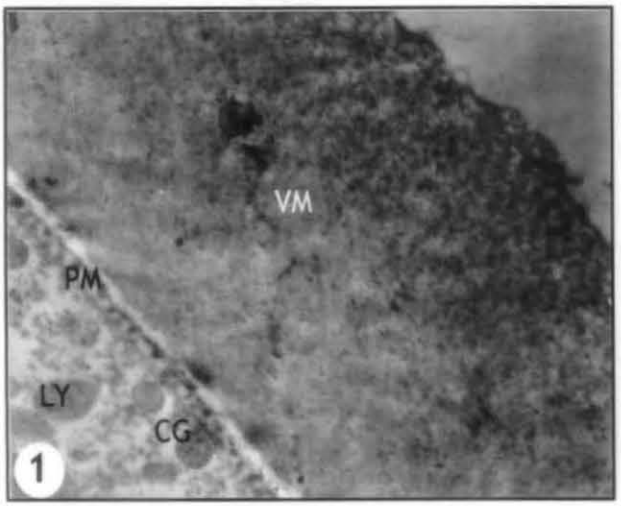
3. Electron micrograph of a degenerating early vitellogenic oocyte (PY- Protein yolk, LY- Lipid yolk, V- Vacuoles, PM- Plasma membrane). X6000.

4. Electron micrograph of a degenerating oocyte with disintegrating vitelline membrane (DVM- Degenerating vitelline membrane, V- Vacuoles, PY- Protein yolk, PM- Plasma membrane, BV, Blood vessel). X15000.

5. Electron micrograph of a degenerating oocyte with disintegrating yolk materials (DYG- Degenerating yolk granules, AV- Autophagous vacuole, PM- Plasma membrane, DVM- Degenerating vitelline membrane). X15000.

6. Electron micrograph of degenerating oocytes in the lumen of partially spawned ovary (DVM- Degenerating vitelline membrane, GBV- Genital blood vessel, V- Vacuoles, Disintegrating yolk, AV- Autophagous vacuoles). X5000.

PLATE XVI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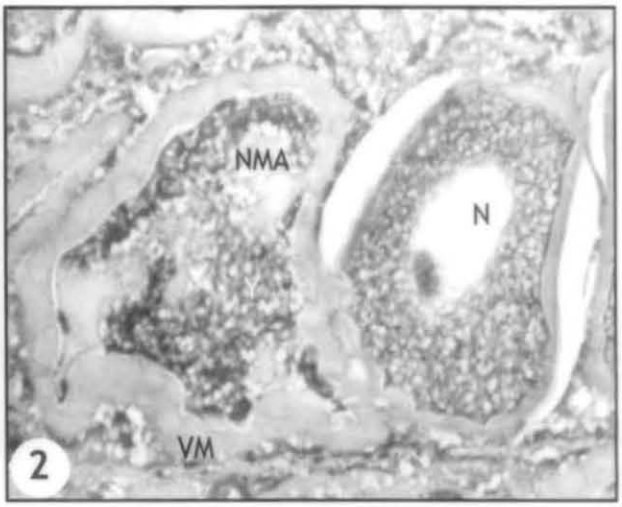
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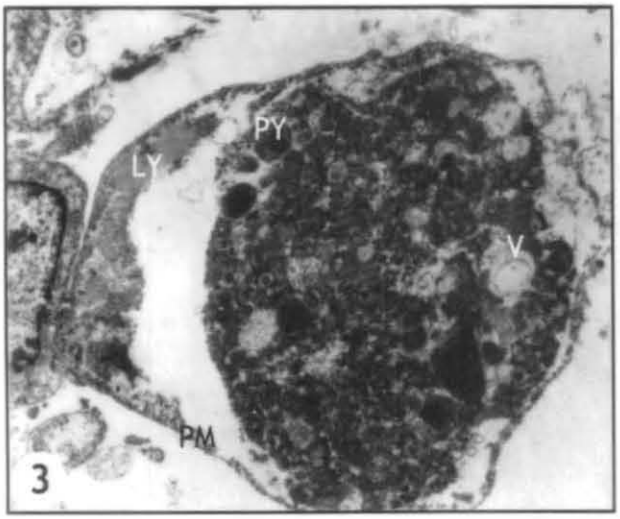
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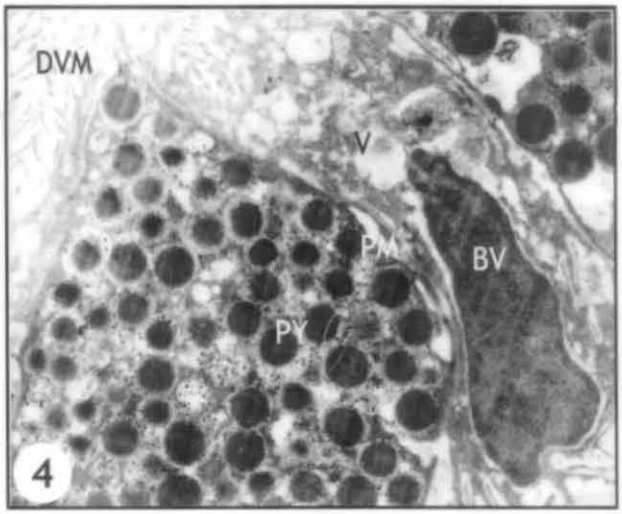
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V
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BV
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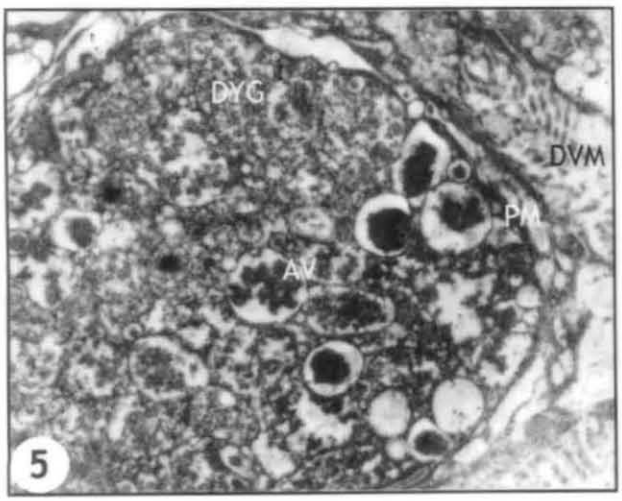
4


DYG
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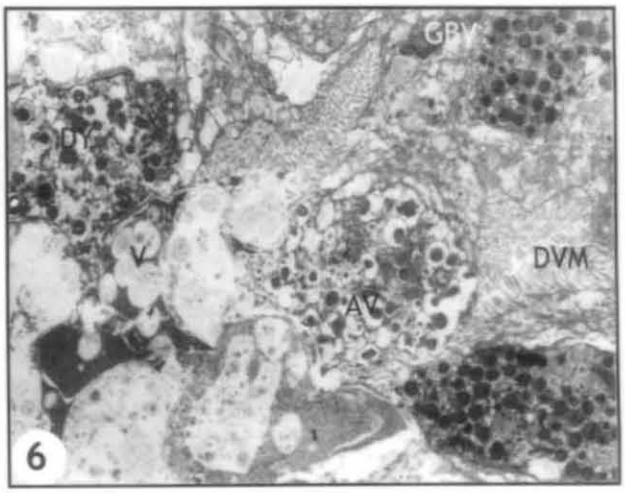
GBV
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6

oocyte (Plate XVI4). Even early vitellogenic oocytes also were seen atretic (Plate XVI3).

5. ANNUAL REPRODUCTIVE CYCLE

Annual reproductive cycle of *Haliotis varia* was studied for a period of 15 months from December 1997 to February 1999, in Tuticorin and Mandapam stations. Monthly samples were collected from these areas and studied for stages of maturity by macroscopic and microscopic examination, as described earlier. The total number of abalones examined to study the annual reproductive cycle at Tuticorin and Mandapam were 618 and 466 respectively.

The gonad index is a convenient representation of the reproductive state of the abalone. The reproductive cycle of *H. varia* using gonadosomatic index for the study period is shown in fig. 1 for Tuticorin and fig. 2 for Mandapam station. Data for males and females at each sample were pooled.

Sampling started in December 1997, which was the end of Northeast monsoon. Almost all the stages of gametogenic activity were found throughout the year of sampling. In the Tuticorin population, the gonad index was highest in January and February and high value maintained from January to April. Completion of spawning was observed in May and immediately after spawning, most of the gonads were in a state of recovery. The resting phase of gonad was very few in the samples collected throughout the year, indicating the onset of gametogenesis immediately after spawning in April-May months. From July to December, the gonad index was low and lowest value observed was in August. The ripe male individuals were present throughout the sampling period; while ripe female animals were restricted to the spawning season only.

In the Mandapam station, the gonad index showed highest value in December indicating the onset of breading season. From this month onwards high values of GSI were maintained. Here also like Tuticorin population, gametogenic activity started immediately after spawning which was evident with the absence of resting phase. Mature male specimens were obtained throughout the sampling period. In April, the GSI was high compared to the preceding month, mainly because of the presence of ripe male gonads in the sample.

The percentage frequency of different stages of gonad in each month for Tuticorin and Mandapam populations are given in tables 13 to 16. The month wise percentage of different stages for the entire sampling period indicates seasonal changes.

5.1. Gonadal condition in Premonsoon (July-September)

In the Tuticorin station during this period, the gonad appeared as less thick but not much transparent over the hepatic gland. Most of the gonads of both the sexes were in early or late maturing condition indicating active gametogensis. In July and August, the occurrence of early maturing and late maturing gonads were 40% and 30% respectively. But 44% of the males were in spent condition. In September, the percentage of partially spawned gonad was 33%, which shows the occurrence of some spawning mainly by the male individuals. Among females early maturing stage dominated in September (70%).

In the Mandapam population, most of the gonads in this period were in the early maturing stage with more numbers in August (52%). This indicates the process of active gametogenesis in this period. 23% of the animals in July were in partially spawned stage, which also may be due to the spawning by the male individuals. In

July 80% of the females were in early maturing condition. Collection was not possible in September from Mandapam station because of the rough sea condition in Gulf of Mannar during the period.

5.2. Gonadal condition in monsoon (October-December)

In the Tuticorin station, the gametogenic activity observed during the previous period became intense and as a result, the percentage of abalones in the late maturing stage increased to 53% in November. In December, 28% of the animals were in partially spawned stage and 41% were in spent stage, which is an indication of onset of spawning. The percentage of spent females was higher (64%) than that of the males (26%). The presence of partially spawned and spent gonads in the sample in December resulted in low GSI value for that month (4.717).

In the Mandapam population also the gametogenic activity was intense in both the sexes with 27% partially spawned and 43% spent gonads in November. So in this stock, the spawning activity would have started by November, one month earlier than that in the Tuticorin stock. In December, 59% of the animals were in the ripe condition, which resulted in the increase in GSI value to 7.76. In October, half of the gonads sampled were in the early maturing condition. But among females 58% of the gonad was in late maturing condition.

5.3. Gonadal condition in Post monsoon (January-March)

In the Tuticorin population, the occurrence of ripe specimen was highest in February with 88%, and in January it was 81%. In February 1999 all the females sampled were in ripe condition. So this period represents the active breeding season for *H. varia* in the Tuticorin station. In March, the percentage of ripe animals was 31% with some animals showed immediate recovery after spawning (23%). As a result of

the rapid growth of germinal cells in the gonad during the previous month, the gonad was full and plumpy, and formed the major part of the conical appendage. The external appearance of gonad was thick and turgid. In 1998 January, the percentage of recovering animals was higher due to the fact that collection was made after the full moon day, by the time most of the animals would have spawned. During this period, the highest GSI values observed were 12.335 in January and 10.847 in February.

In the Mandapam station, the percentage occurrence of ripe gonad was 59% and 48% in January and February respectively. The presence of partially spawned and spent stage animals also was higher, which indicates spawning. For Mandapam population, breeding season started from December and continued to January and February. The presence of ripe males was more in the above months with 68% and 63% respectively. In March, only 3% of the gonad was ripe with 62% showing active recovery immediately after spawning. The GSI values also were higher in January and February with 6.04 and 7.00 respectively.

5.4. Gonadal condition in summer (April-June)

In the Tuticorin station, some ripe individuals were present in April and May (23% and 21% respectively). But most of the animals were in a stage of recovery as evident by the presence of early maturing (32%) and late maturing (34%) gonads in June and May respectively. 23% of indeterminate animals were present in April, which was mainly due to the presence of smaller animals in the sample. In April and May, 35% and 38% of males respectively were in ripe condition. In June, the percentage of spent animals was 24%, which were not yet recovered for the next gametogenic cycle.

In the Mandapam population, during the summer period, the percentage of early and late maturing animals was higher than the other stages. So the active gametogenesis takes place during this period in Mandapam population. The collection could not be done in May due to rough sea condition in the Gulf of Mannar. In April, the presence of early maturing and late maturing animals were 43% and 30% respectively and in June they were 37% and 30% respectively. In April the percentage of early maturing female was 64%.

The process of cytolysis and resorption were observed in months just after the completion of spawning. Thereafter, instead of entering into a resting phase, the abalones showed active recovery in the proceeding months. From April onwards, the gametogenic activity was initiated in both the populations and the abalones entered into the active phase of gonad development. In December, January and February, as a result of the rapid growth of germinal cells, the gonads attained full maturity. From February to June, mild spawning was observed by the presence of large percentage of partially spawned and spent gonads. A small peak of spawning was evident in the premonsoon period due to the presence of partially spawned and spent gonads.

It may be seen from the foregoing account that *H. varia* of Tuticorin and Mandapam stations follow a more or less definite pattern of annual reproductive cycle with a short breeding period extending from December to March in Tuticorin and November to February in Mandapam. The gonadal changes were cyclical with well-defined phases of gametogenesis such as maturation, ripening, spawning and regression. Gametogenesis or maturation commences from March and April. Ripening takes place mainly in December- January. The spawning activity continues

Table 13. Percentage distribution of different maturity stages of *H. varia* during the study period at Tuticorin station

Month	Percentage of maturity stages					
	Stage I	**Stage II**	**Stage III**	**Stage IV**	**Stage V**	**Stage VI**
December	45	41	10	3	0	0
January	48	21	24	0	3	3
February	7	7	57	17	3	10
March	23	10	31	10	3	23
April	21	16	23	14	2	23
May	17	34	21	10	7	10
June	32	20	8	8	24	8
July	40	8	8	12	24	8
August	30	22	0	4	17	26
September	19	19	15	33	15	0
October	30	53	0	0	10	7
November	32	25	0	29	14	0
December	10	17	3	28	41	0
January	0	3	81	5	3	8
February	0	4	88	8	0	0

Table 14. Percentage distribution of different maturity stages of *H. varia* during the study period at Mandapam station

Month	Percentage of maturity stages					
	Stage I	**Stage II**	**Stage III**	**Stage IV**	**Stage V**	**Stage VI**
December	13	9	59	13	3	3
January	37	10	33	7	0	13
February	45	17	10	10	7	10
March	62	17	3	3	14	0
April	43	30	13	13	0	0
May	-	-	-	-	-	-
June	37	30	7	10	10	7
July	34	6	17	23	17	3
August	52	10	3	10	13	13
September	-	-	-	-	-	-
October	50	42	0	0	4	4
November	17	3	10	27	43	0
December	17	13	40	20	7	3
January	3	13	59	19	6	0
February	21	10	48	3	14	3

Table 15. Monthly distribution of male and female maturity stages of *H. varia* during the study period at Tuticorin station.

Months	Percentage of maturity stages									
	Male					Female				
	I	II	III	IV	V	I	II	III	IV	V
December	32	37	16	5	11	60	40	0	0	0
January	20	10	70	0	0	68	26	0	0	5
February	0	12	65	18	6	20	0	60	20	0
March	13	13	40	27	7	38	15	46	0	0
April	6	24	35	29	6	50	8	33	8	0
May	15	15	38	15	15	29	57	7	7	0
June	18	9	18	18	36	57	29	0	0	14
July	10	0	10	30	50	71	12	12	0	6
August	22	22	0	11	44	70	30	0	0	0
September	7	13	10	40	30	47	29	12	12	0
October	29	50	0	0	21	36	64	0	0	0
November	13	25	0	50	13	57	21	7	0	14
December	5	21	5	42	26	27	9	0	0	64
January	0	7	79	14	0	0	12	84	0	4
February	0	11	67	22	0	0	0	100	0	0

Table 16. Monthly distribution of male and female maturity stages of *H. varia* during the study period at Mandapam station.

Months	Percentage of maturity stages									
	Male					Female				
	I	II	III	IV	V	I	II	III	IV	V
December	0	16	74	11	0	33	0	42	17	8
January	26	16	47	5	5	75	0	13	13	0
February	31	31	15	15	8	69	8	8	8	8
March	53	33	7	7	0	100	0	0	0	0
April	25	31	19	25	0	64	29	7	0	0
June	38	19	13	13	19	42	50	0	8	0
July	0	13	33	27	27	61	0	6	22	11
August	33	8	8	17	33	80	13	0	7	0
October	33	58	0	0	8	73	27	0	0	0
November	6	6	6	24	59	31	0	15	31	23
December	6	12	59	18	6	33	17	17	25	8
January	0	11	68	21	0	8	15	46	15	15
February	6	13	63	0	19	42	8	33	8	8

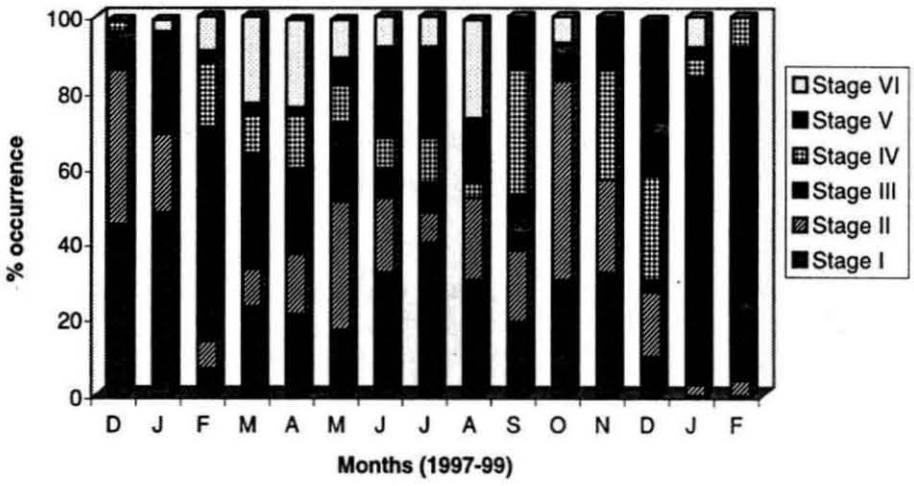


Fig.15. Percentage occurrence of different maturity stages of *H. varia* at Tuticorin station during the study period.

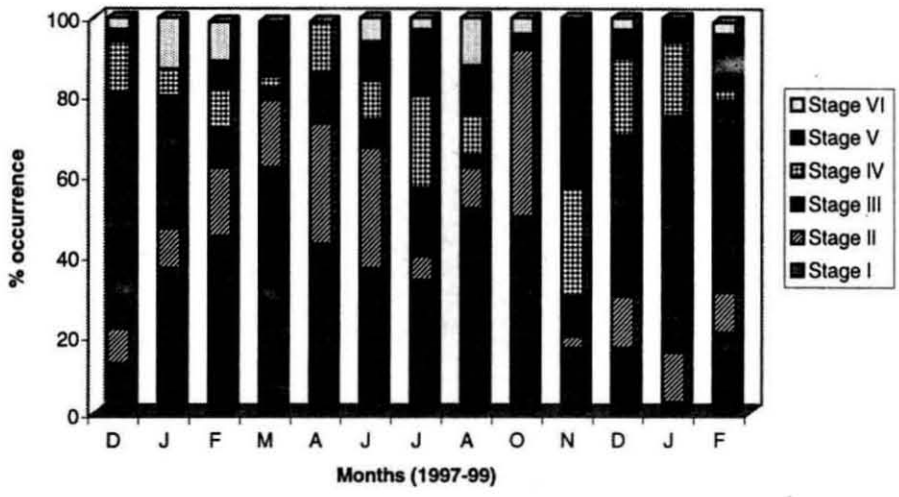


Fig. 16. Percentage occurrence of different maturity stages of *H. varia* at Mandapam station during the study period.

from December to March and the animals enter into an active recovery phase thereafter.

6. HYDROGRAPHIC CONDITIONS OF COLLECTION STATIONS

The data on different hydrographical parameters of the abalone habitat were collected from Tuticorin and Mandapam stations in the study for a period of 15 months from December 1997 to February 1999. The monthly values of all the parameters at each station studied are given in tables 17 and 18. Correlation matrix of the various hydrographic parameters and gonad index during the study period were calculated and shown in table 19.

6.1. Temperature

The temperature recorded at the two stations during the study period is shown in fig. 17 and 18. The monthly atmospheric temperature and water temperature did not vary much over the period. In the Tuticorin station, the monthly atmospheric temperature fluctuated between 23.5 °C (January, 1999) and 33.0 °C (April, 1998 & 99) and the water temperature fluctuated between 24.5 °C (January, 1999) and 31.5 °C in April 1999. At Tuticorin, the mean annual range of variation being 8.1 °C in atmospheric temperature and 4.2 °C in water temperature. At Mandapam, the annual range of variation was 6.2 °C in atmospheric temperature and 4.1 °C in water temperature.

The pattern of oscillation seen in the atmospheric temperature at Tuticorin station was a bimodal with two peaks and two depressions corresponding to the months of April and September and January and June. The pattern for Mandapam station was unimodal with one peak (April-July) and one depression (January). The

pattern of oscillation in water temperature also bimodal in Tuticorin station and unimodal in Mandapam station.

6.2. Salinity

The salinity data recorded for the entire study period are shown in fig. 19 and 20. Unlike temperature, the salinity showed monocyclic condition in its annual variation in both the stations. In Tuticorin station, the salinity was high during the period of Southwest monsoon (June-September) and low during the period of Northeast monsoon (December-January). The same pattern is followed in the Mandapam station also. At Tuticorin, the monthly salinity ranged between 30 ppt (December and January) and 37 ppt (August 1998). In the Mandapam station, the highest salinity recorded was 36 ppt (October, 1998) and the lowest was 30 ppt (March, 1998).

The pattern of oscillation seen in the salinity values of Tuticorin and Mandapam stations were unimodal with one peak and one depression corresponding to the months of August and February in Tuticorin and October and March in Mandapam.

6.3. pH

The pH value of the Tuticorin and Mandapam stations did not show much fluctuation during the 15-month study period. At Tuticorin station, it showed maximum value of 8.58 in May 1998 and the minimum value was in February 1999 (8.15). At Mandapam, the pH value fluctuated between 8.1 in March 1998 and 8.69 in October 1998. The monthly variations in the pH value of both the stations are given in the fig. 21 and 22.

6.4. Dissolved oxygen

The variation in the range of dissolved oxygen value was much more between the Tuticorin and Mandapam stations. In the Tuticorin station lower values were observed in most of the months of collection with the minimum value of 2.517 mg/l in July and the maximum of 5.836 mg/l in January 1998. In the Mandapam station, the dissolved oxygen values were always higher than 5 mg/l and ranged between 5.149 mg/l in April and August months and 5.836 mg/l in June. The monthly variations in dissolved oxygen content at Tuticorin and Mandapam are shown in fig. 23 and 24.

Table17. Monthly values of various hydrographic parameters recorded at Tuticorin during the study period

Months	Atmospheric Temperature °C	Water Temperature °C	Salinity ppt	pH	Dissolved oxygen mg/l
December '97	26.5	27.0	30	8.30	5.04
January '98	25.5	26.0	30	8.25	5.84
February	27.0	28.0	31	8.27	2.86
March	31.0	30.0	30	8.25	4.81
April	33.0	31.0	33	8.48	3.10
May	32.0	30.0	34	8.58	3.55
June	28.0	27.0	35	8.38	4.12
July	28.5	27.0	35	8.34	2.52
August	30.5	28.0	37	8.36	4.58
September	31.5	28.0	35	8.50	3.90
October	28.0	28.0	35	8.41	4.35
November	27.0	28.5	36	8.39	3.89
December	25.0	27.0	34	8.41	4.43
January '99	23.5	24.5	31	8.46	5.15
February	25.0	26.5	33	8.15	5.72

Table18. Monthly values of various hydrographic parameters recorded at Mandapam during the study period

Months	Atmospheric Temperature °C	Water Temperature °C	Salinity ppt	pH	Dissolved oxygen mg/l
December '97	26.5	28.0	32	8.50	5.63
January '98	25.5	26.5	31	8.30	5.43
February	27.0	28.0	31	8.20	5.23
March	29.0	30.0	30	8.10	5.04
April	31.0	32.0	33	8.54	5.15
June	31.5	30.5	34	8.68	5.84
July	29.5	31.0	35	8.26	5.63
August	29.5	29.0	35	8.66	5.15
October	28.0	27.0	36	8.69	5.72
November	28.0	29.5	32	8.66	5.74
December	26.0	27.5	33	8.52	5.52
January '99	25.0	26.0	33	8.45	5.31
February	25.5	27.5	34	8.62	5.51

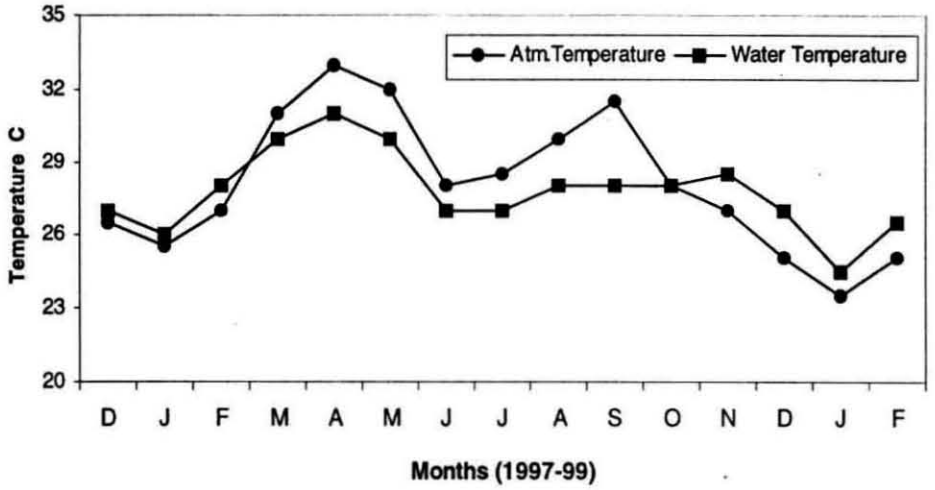


Fig.17. Monthly variations in atmospheric temperature and water temperature at Tuticorin station during the study period

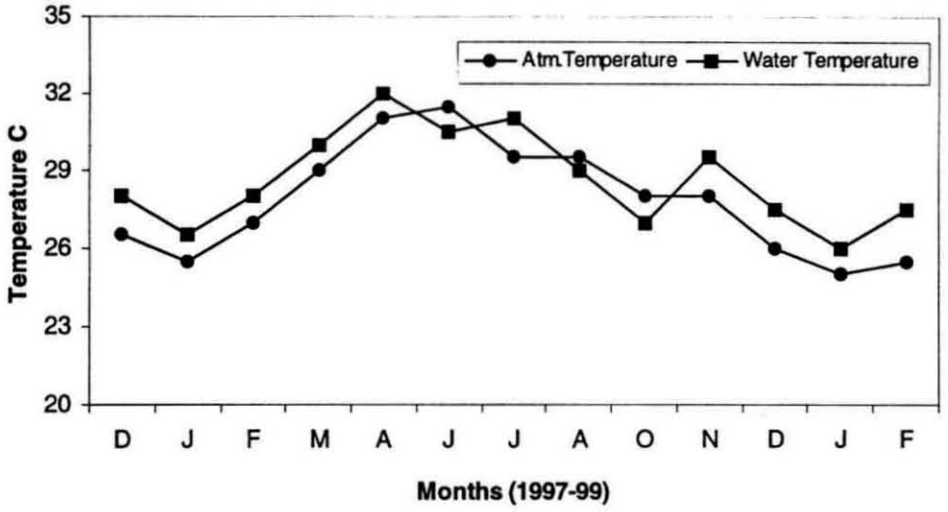


Fig. 18. Monthly variations in the atmospheric temperature and water temperature at Mandapam station during the study period.

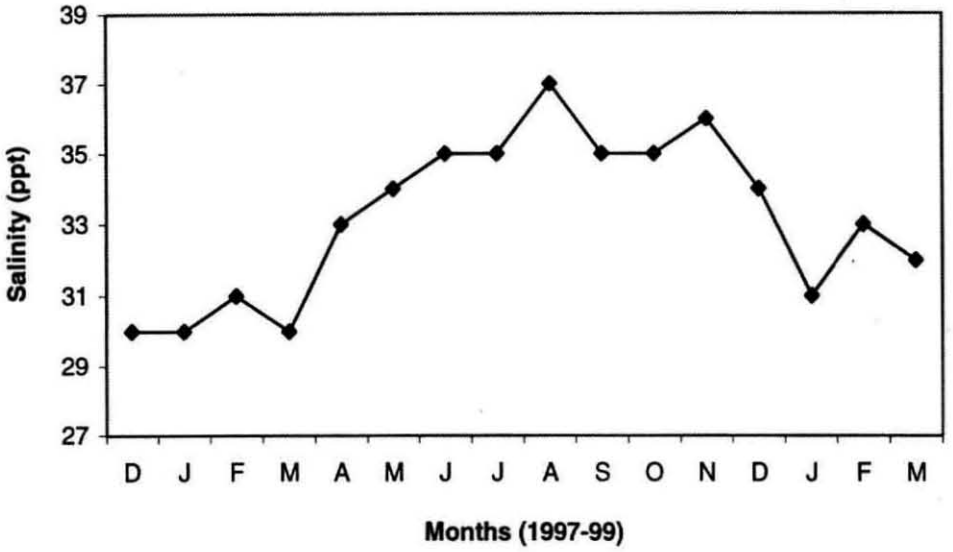


Fig.19. Monthly variation in the salinity at Tuticorin station during the study period.

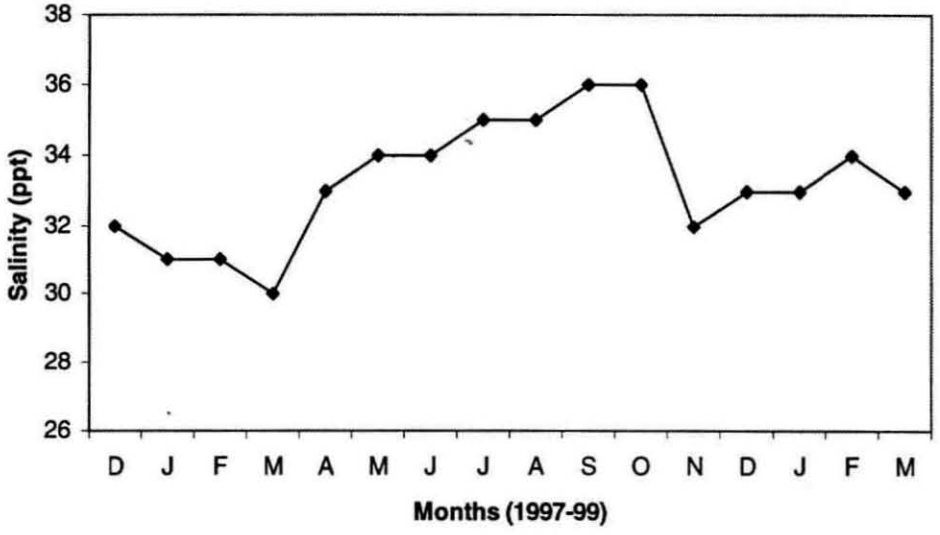


Fig. 20. Monthly variation in the salinity at Mandapam station during the study period

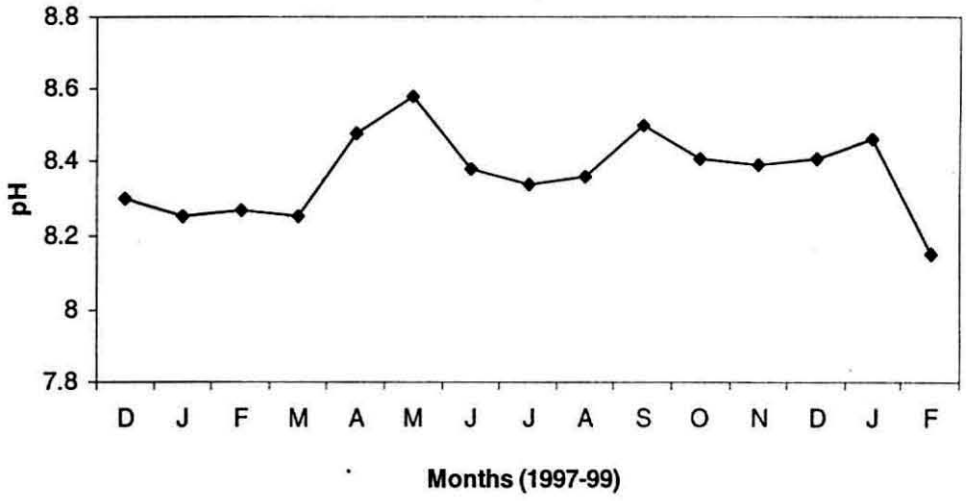


Fig. 21. Monthly variation in the pH value at Tuticorin station during the study period

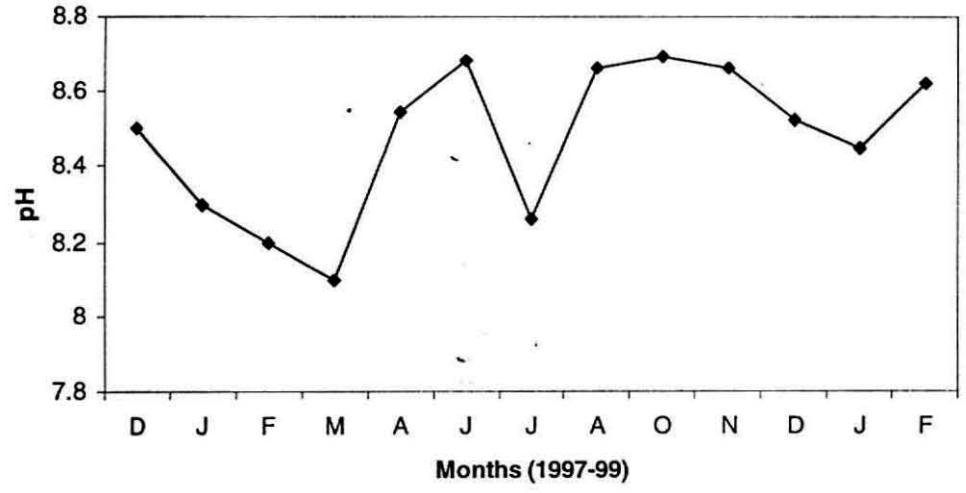


Fig. 22. Monthly variation in the pH value at Mandapam station during the study Period

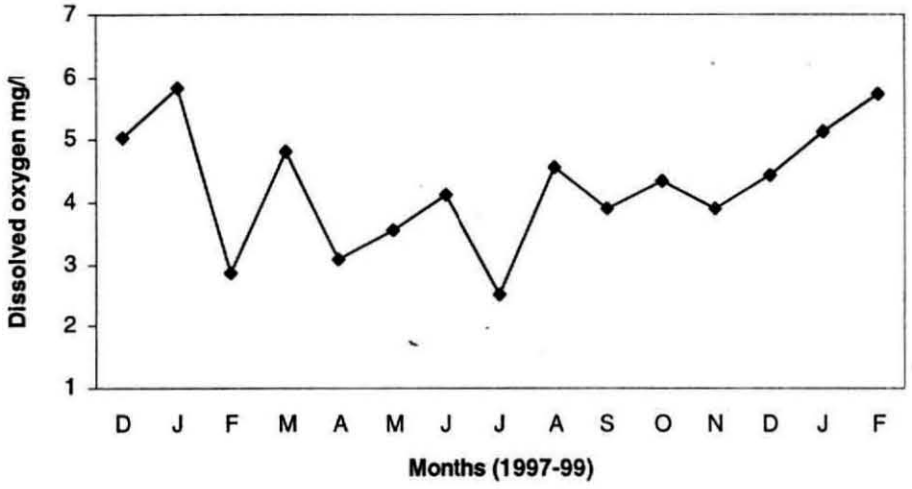


Fig. 23. Monthly variation in dissolved oxygen content at Tuticorin station during the study period

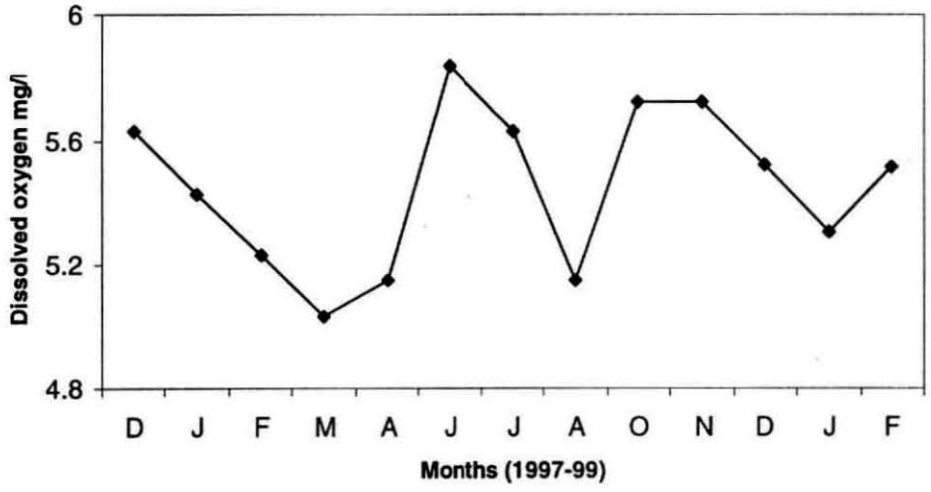


Fig. 24. Monthly variation in dissolved oxygen content at Mandapam station during the study period

Table 19. Correlation matrix of the hydrographic parameters and the gonadosomatic index during the study period at Tuticorin and Mandapam stations.

A. Tuticorin

	Salinity	Water temperature	Atmospheric temperature	pH	Dissolved oxygen	Gonadosomatic index
Salinity	1					
Water temperature	0.172364	1				
Atmospheric temperature	0.293301	0.867916	1			
pH	0.412221	0.311639	0.457799	1		
Dissolved oxygen	-0.38181	-0.50262	-0.52613	-0.44093	1	
Gonadosomatic index	-0.48465	-0.25677	-0.30899	-0.17877	0.156195	1

B. Mandapam

	Salinity	Water temperature	Atmospheric temperature	pH	Dissolved oxygen	Gonadosomatic index
Salinity	1					
Water temperature	0.063576	1				
Atmospheric temperature	0.272947	0.887462	1			
pH	0.64503	-0.03644	0.153904	1		
Dissolved oxygen	0.418212	-0.08313	-0.00459	0.507793	1	
Gonadosomatic index	-0.22046	-0.33636	-0.55812	-0.03956	0.05572	1

r values above 0.4 are significant at 5% level.

DISCUSSION

In *H. varia*, the sexes are separate. Gonad is a single conical colourful organ lying over the digestive gland and covering part of the stomach. So the conical appendage, which is situated on the right side of the foot, comprises the digestive gland, gonad and a part of the stomach. When fully ripe, female gonad becomes dark bluish or greenish in colour. The ripe male gonad is bright creamy in colour.

Like other abalone species, *H. varia* also is a broadcast spawner ie., at spawning, the gametes are released into the environment and fertilisation is external. The mature gonad of broadcast fertilisers constitutes a large part of the body weight; for *H. cracherodii* 20% of the soft parts (Webber and Giese, 1969); for the Pismo clam 25% of the total weight (Giese *et al.* 1967) and for sea stars, 10% of the total body weight (Mauzey, 1966). For *H. varia*, ripe gonad constituted around 15% of the soft body weight.

Light microscopic studies during the present investigation revealed that the gonad of *H. varia* is similar to the description given by Crofts (1929) for *H. tubercualata*, Newman (1967) for *H. midae* and Young and DeMartini (1970) for *H. rufescens*. The gonad of *H. varia* consists of a large lumen, which is bounded by germinal epithelium with a base of connective tissue. The outer gonadal wall is similar in both the sexes and it is a single glandular epithelium covered by a thin layer of cuticle. Strands of underlying connective tissue grow outward in thin folds and form as trabeculae between overlying digestive gland and integument. Germinal tissues are present, lining the inner gonadal wall as well as the parallely running trabeculae. The

role of the trabeculae is mainly supportive and acting as a base for the germinal tissue in *H. varia* as reported by Wood and Buxton (1996) in *H. midae*.

The section through the ovary and testis are somewhat similar except that the eggs are exclusively and tightly packed in the ripe ovary. In the ovary, most trabeculae end freely in the lumen rather than attach to the wall of digestive gland. Newman (1967) reported that this might be for allowing free movement of eggs during spawning. The cuboidal primary oogonia with around 5 µm diameter, which is produced by the germinal epithelium, can be seen near the inner wall of the ovary. In the ripe testis, the section of vertical connective tissue tubes can be seen in the cross section. The outer surfaces of connective tissue tubes are surrounded initially by squamous germinal epithelium, which later becomes cuboidal. Spermatogonia are produced by the germinal epithelium and can be seen surrounding the connective tissue tubules.

In the ovary and testis, the gametes are arranged in a graded manner. Immature gametes are found more near the periphery of the gonad. The mature eggs and spermatozoa are distributed abundantly in the lumen near the digestive gland.

Different criteria such as percentage of gonad covering the digestive gland, colour and shape of gonad, gonadosomatic index and ova diameter, were used by various workers in order to classify the maturity stages in abalone. Tomita (1967) classified the maturity stages as premature, mature, spawning, spent and recovery. Ault (1985) called them as preproliferative, proliferative, new stalk, old stalk and free. In *H. rufescens*, Giorgi and DeMartini (1977) called them active, ripe, partially spawned, spent and necrotic. However, the above classifications were developed primarily for the temperate abalones, which have a definite spawning season within

which the stages of maturity are fairly uniform throughout the population at any one time. Majority of the tropical species are having prolonged breeding season. In these species almost all the maturity stages may be available throughout the year.

Wood and Buxton (1996) classified the maturity stages of *H. midae* into six, which were immature, ripe, spawning, early recovery and active recovery. In the present study, the maturity stages assigned to *H. varia* also were six, which were early maturing, late maturing, ripe, partially spawned, spent and indeterminate stage. The above classification was based on the general appearance of gonads, size, shape, colour and ova diameter measurements. The early maturing and late maturing stages correspond to the two recovery stages mentioned by Wood and Buxton (1996). Morphologically the early maturing ovary was a thin grayish sheath around the massive digestive gland and flaccid in nature. The ovary in this stage was filled with primary oocytes and most of them were attached to the trabeculae by a stalk. This stage can be compared to the new stalk stage suggested by Ault (1985). The colour of the early maturing testis was pale orange and the gonad was covering only 30% of the conical appendage.

In the late maturing ovary, the gametogenic activity becomes more intense and apart from previtellogenic oocytes, plenty of early vitellogenic oocytes with diameter ranging from 50 to100 μm can be seen. In this stage, the oocytes become teardrop shaped and this corresponds to the old stalk stage mentioned by Ault. The ovary becomes turgid and colour changed to blue gray. The testis in this stage is turgid and orange cream covering 50% of the conical appendage. It is characterized by the proliferation of spermatocytes and spermatids. They were found to be arranged in a graded manner in the testis, from the periphery of connective tissue tubules.

The ripe ovary of *H. varia* is turgid, cylindrical and dark blue in colour. It contains exclusively mature eggs, which are tightly packed around a narrow strand of trabeculae. An ovary is classified as ripe when it contains more detached oocytes than attached oocytes. There was no gelatinous matrix covering the oocyte observed in the ovary of *H. varia*, as those found in the ripe ovary of *H. midae* by Newman (1967). The eggs are found to be loosely packed near the digestive gland. The ripe gonad of H. *varia* at Phuket was reported as creamy white in males and dark green in females (Bussarawit *et al.*, 1990).

The ripe testis of *H. varia* can easily be distinguished by the bright cream colour and turgid, bulging and cylindrical appearance. In crossection, little or no digestive gland material can be seen in the conical appendage. The description of ripe ovary and testis of *H. varia* are similar to the description given for *H. midae* by Newman (1967) and Wood and Buxton (1996).

The spent stage gonads of *H. varia* are very much loose and collapsed. In the ovary, some unspawned mature oocytes can be seen. In the spent testis, residual spermatozoa along with some darkly stained spermatids are discernible.

In most broadcast fertilizers, after spawning, the gonad enters a 'resting stage' in which gametes are absent (Loosanoff, 1962). For *H. varia*, such resting phase was found but only for a short period and immediately after that gametogenesis was initiated. For *H. cracherodii*, however, no period of gonad quiescence was found (Webber and Giese, 1969).

The reproductive cycle is reflected by pronounced variation in gonadal size. When assessing gonadal activity, animals of different sizes are frequently sampled and it is generally assumed that gonadal weight depends on animal size and stage of

gonadal development (de Vlaming *et al.*, 1982). To quantify the spawning season, several researchers have developed methods to standardize the gonadal development by comparing the gonad size to a measurement (ie., shell length, size of digestive gland or body weight) of the animal that is independent of gonad size (Ault, 1985; Young and DeMartini, 1970.; Hahn, 1981). In *H. cracherodii*, Webber and Giese (1969) calculated the GSI as the weight of the gonad relative to the weight of the soft parts. In the present study, the GSI was calculated as suggested by Webber and Giese (1969).

Gonad index varies with the maturity stage of the abalone. For *H. varia*, high value of GSI obtained in the ripe stages both in Tuticorin and Mandapam stations. The lowest values obtained in the indeterminate stage of the gonad. Gonad index gradually increases from stage I to stage III and then declines indicating spawning. From this, it is clearly evident that GSI is having a positive correlation with the maturity condition of the gonad of *H. varia*. The GSI values observed for Mandapam station always lesser than that for the Tuticorin animals. This may be due to the fact that the mean shell length of abalones from Mandapam is less than that from the Tuticorin station.

In the present study, highest values of mean monthly gonad index were observed during the breading season of *H. varia*. Boolootian *et al.* (1962) found that monthly samples of gonad indices of black abalones showed seasonal variation in the Pacific Grove area. High values of gonad indices coincided with the breading season.

Fretter and Graham (1964) report that in dioecious molluscs, females tend to be more numerous than males and this is especially evident in older populations. Such a tendency was not found in *H. midae* by Newman (1967). Instead a

preponderance of males has been recorded in *H. tuberculata* (Foster, 1962; Stephenson, 1924). In *H. varia*, in both the stations, the male female ratio was almost equal and at Tuticorin station, females slightly outnumbered males but not significantly.

The sex ratios of other haliotids have been reported. A large sample of *H. tuberculata* was examined by Crofts (1937), who found that sexes were almost equally distributed. Sinclair (1963) examined 598 *H. iris* and found 352 males. The sex ratio was not significantly different from 1:1 in either *H. australis* or *H. iris* (Poore, 1973). Most studies in other species of abalone have reported 50:50 sex ratios (Newman, 1967; Webber and Giese, 1969; Young and DeMartini, 1970; Quayle, 1971; Hayashi, 1980).

In several marine invertebrates, the stage of sexual maturity has been shown to be a function of size (Wenner *et al.*, 1974). According to Wood and Buxton (1996), in *H. midae*, an individual was classed as mature if it contained mature gametes, defined as spermatozoa in males and vitellogenic oocytes in females. The size at maturity for *H. midae* at Great Fish Point was 55.2 mm (Wood and Buxton, 1996). Newman (1967) found that at the shell breadth range of 8-8.9 cm more than 60% of the individuals of *H. midae* were sexually mature. In both the cases the maximum shell length of *H. midae* in the population was more than 150 mm. But *H. varia* is a small tropical abalone with a maximum shell length less than 70 mm.

In the present study, it was found that male and female individuals of *H. varia* in the same population attained sexual maturity at different sizes. More than 50% of males in the Tuticorin population of *H. varia* can be expected to have gametes at a shell length range of 18 to 20 mm but that for females was 22 to 24 mm. In the

Mandapam population size of sexual maturity observed among the males was little higher than that of Tuticorin population; at the shell length range of 20 to 22 mm. But females attained sexual maturity at the same shell length range as the Tuticorin population. As the minimum exploitable size limit of abalones is based on the size at first maturity, the difference could have importance for management to ensure maximum sustainable utilization of the species in different areas of its distribution. At Andaman sea coast of Thailand, the minimum size of individual *H. varia* having mature eggs reported was 17.3 mm (Bussarawit *et al.* 1990).

Since the success or failure of a cultivable species largely depends on its spawning potential, the knowledge of fecundity becomes extremely important from the viewpoint of successful management and exploitation of its fishery. In broad terms, fecundity may be defined as the number of eggs produced by an individual during its lifetime (Lowe-McConnell, 1975). However, most authors define fecundity in more practical terms as the total number of eggs present in the ovary prior to spawning (Newman, 1967; Poore, 1973).

In the present investigation, the number of eggs calculated in individual *H. varia* ranged from 15,160 in the animal with a shell length of 26.66 mm to 2,75,663 at a shell length of 48.04 mm. The fecundity reported by Bussarawit *et al.* (1990) for *H. varia* at Phuket ranged from 96,747 to 3,505,230 at the shell length of 17.3 and 41.8 mm respectively. So the estimates obtained here are lesser than that obtained by the above authors. The fecundity is found to vary among the individuals of *H. varia*. Variability in the egg production in *H. midae* has been reported by Newman (1967) and Wood and Buxton (1996).

Abalone can be expected to produce large numbers of eggs, and Fretter and Graham (1964) recorded that *H. tuberculata* carried 10^4 eggs but no animal size was associated with this value. The fecundity of the abalone known to vary between individuals, between populations and even between years (Tutschulte and Connell, 1981; Ault, 1985, McShane *et al.*, 1986; Shepherd, 1987) due to differences in food availability. In general, the fecundities in the tropical abalones are found to be lesser than that in the temperate abalones, which are larger in size than the former.

The number of eggs calculated in individual *H. iris* ranged from 1,286 at 68 mm to11,253,000 at 155 mm (Poore 1973). The fecundity estimate reported was 1,400,00 eggs in *H. seilboldii* (Ino, 1952). The fecundity of Spanish abalone *H. coccinea canariensis* that grows to a maximum size of 8 cm in shell length was found to range between 11,000 and 70,000 (Pena, 1986).

In the present study, the fecundity bore a curvilinear relationship with shell length (L) and linear relationship with total weight (W) and gonad weight (G), with high correlation coefficients in all the three relationships between fecundity and body parameters. Newman (1967), Poore (1973) and Hayashi (1980) all reported that the number of eggs increases exponentially with increase in the length of the abalone, a feature recorded for *H. roei* also by Wells and Keising (1989). Ault (1985) reviewed the fecundity of abalone and noted that a linear relation with weight is found for many species. This is expected because proliferation of oocytes, strictly a surface phenomenon, should be related to gonad volume.

One acknowledged function of molluscan digestive gland is the resorption of extracellularly digested food (Van Weel, 1961), giving this organ short-term storage capability. The size of the digestive gland and its importance as a storage organ are

dependant on the incoming food supply (Thompson *et al.*, 1974). A negative correlation has been reported between the digestive gland and gonad indices over the reproductive periods of the Massachusetts and North Carolina scallop populations (Sastry, 1970). Barber and Blake (1983) concluded that the reproductive energy of the above populations is being supplied from the nutrients in the digestive gland. The transfer of ^{14}C leucine from the digestive gland of *Argopecten irradians* during oocyte development has been demonstrated (Sastry and Blake, 1971).

The monthly changes in the hepatosomatic index (HSI) for *H. varia* were calculated for both Tuticorin and Mandapam populations. HSI for different stages also were determined for both the stations. In all the cases, HSI was lowest in stage III when the GSI was highest. The HSI was lower in stages I and II in most of the cases. Lower HSI values were obtained during the breeding season of *H. varia* in both the stations, when the GSI were higher. Similar observations were reported by Boolootian *et al* (1962) and they noticed that the hepatic index was negatively correlated with gonad index both in *H. cracherodii* and in *H. rufescens*.

Ova diameter study is a useful tool to know the spawning period and spawning frequency in molluscs. Based on the measurement of the ova in the ripe ovary evidence of the duration of spawning in an animal ie., whether the spawning period is short and definite or long and indefinite, could be obtained. The ova diameter frequency polygon of *H. varia* showed a decreasing trend in the percentage of immature ova with respect to the maturing group as maturation advanced and immature ova were absent in the beginning of spawning (ripe stage). The ripe stage ovary of *H. varia* contained a clear and well-differentiated group of eggs with a diameter mode at 171 µm and a diameter range of 137 to 228 µm. All the eggs were

found to be vitellogenic and they were exclusively occupied in the ovarian lumen. From this it is clearly evident that for *H. varia* the spawning period is short and definite. The diameter of the oocytes gradually increased from stage I to stage II. The stage IV (partially spawned) ovary contained oocytes with same diameter range as the ripe ovary. In the stage II ovary, the early vitellogenic oocytes started appearing and almost all the oocytes when grown to a diameter of 57 μm entered the phase of vitellogenesis.

Electron microscopic studies on the testis of *H. varia* revealed that a series of steps are involved in the formation of mature spermatozoon which included spermatogenesis and spermiogenesis. During spermatogenesis, the cells of the germinal epithelium of the testis give rise to spermatogonia and then to spermatocytes. During spermiogenesis, the spermatids and spermatozoa are formed from the spermatotocytes. The literature on the spermatogenesis in haliotids is scarce. Instead, the morphology of the mature spermatozoa of different *Haliotis* sp. have been studied in detail using transmission electron microscopy (Lewis *et al.*, 1980; Sakai *et al.*, 1982; Shiroya and Sakai, 1984; Healy *et al.*, 1998) and Scanning Electron microscopy (Liu *et al.*, 1987). Hence the early stages of spermatogenesis of *H. varia* could not be compared with that of other haliotids. But it is found to be more or less similar with many other molluscan species studied. More similarity was observed to *Mytilus* spp. (Hodgson and Bernard, 1986), *Helcion pellucidus* (Sousa and Oliveira, 1994), *Rapana venosa* (Chang and Kim, 1997) and patellid limpets (Hodgson and Bernard, 1988) with minor differences.

The spermatogonia of *H. varia* were more or less oval and the nucleus contained clumps of electron dense chromatin, which were associated with the inner

nuclear membrane. The nucleus was prominent and occupied the majority of the cell. Similarly, in patellid limpets, Hodgson and Bernard (1988) reported that the spermatogonial nucleus contained electron dense clumps of chromatin that were found to be associated with the inner nuclear membrane. More spermatogonial cells were observed around the connective tissue tubes of testis in *H. varia*.

The primary spermatocytes were derived from the spermatogonial cells by mitotic division. As in all other molluscs studied, the spermatocytes of *H. varia* were smaller than the spermatogonial cells. In contrast to the spermatogonial cells the chromatin was found to be dispersed in the nucleus of spermatocytes. The cytoplasm of spermatocytes contained the same type of cell organelles such as mitochondria, Golgi bodies, free ribosomes etc.. Apart from that, dark round proacrosomal vesicles were also discernible in the cytoplasm of the primary spermatocytes. During the formation of mature spermatozoon, they fuse to form the acrosomal vesicle and occupy the anterior part of the spermatozoon.

Sousa and Oliveira (1994) reported that the formation of proacrosomal vesicles in the primary spermatocytes of *H. pallucidus* constituted the first description in Patellidae. In contrast, the proacrosomal vesicles have been reported to be formed only in spermatids in other patellidae (Hodgson and Bernard, 1988). The presence of proacrosomal vesicles in the primary spermatocyte stage was also observed in several bivalves (Longo and Anderson, 1969; Hodgson and Bernard, 1986; Hodgson *et al.*, 1990). Healy *et al.* (1998) observed these vesicles in the early spermatids of *H. laevigata* but suggested their production might have commenced in spermatocytes as noted by Sousa and Oliveira (1994) in *H. pellucidus*.

The second maturation of secondary spermatocytes resulted in the formation of two spermatids. Three stages of spermatids were observed in *H. varia* depending on the extent of chromatin condensation, cytoplasmic volume and size. The nucleus of early spermatids was ellipsoidal and surrounded by a thin layer of cytoplasm. Numerous proacrosamal vesicles were observed in this stage. In the mid spermatid, the nucleus becomes more condensed and the cytoplasm was a narrow rim around the nucleus. The nuclear diameter also reduced than that of the early spermatid. The nucleus of the late spermatid was much condensed and smaller and the cytoplasm was lost. The proacrosomal vesicles migrated to the anterior end of the spermatid. The smaller mitochondria fused to form four large mitochondria. Similar observations were noted in the spermatids of *H. laevigata* by Healy *et al.* (1998).

The use of spermatozoon ultrastructure for phylogeny and taxonomy is now widely accepted and has also been related to different aspects of reproductive biology (Franzen, 1970, 1983). Further, Hodgson and Bernard (1988) states that each species of patellid limpets possesses a sperm with a unique form suggesting that sperm morphology can be a valid and valuable taxonomic character. Externally fertilizing spermatozoa of gastropods are also reported to exhibit substantial differences in acrosomal form from the order down to the species level (Healy *et al.*, 1998).

The mature spermatozoon of *H. varia* described in this study was a modified type and confirmed to the general pattern noted for *H. laevigata* by Healy *et al.* (1998). The nucleus of spermatozoa was elongate and barrel shaped. The acrosomal vesicle was a conical and membrane bound body. The results of the present study have shown that the spermatozoa of *H. varia* are more similar to those of East Asian

species, *H. diversicolor supertexta* (Gwo *et al.*, 1997; Shiroya and Sakai, 1992). In this species also the nucleus is barrel shaped and shorter. In contrast, spermatozoa of the Japanese species *H. discus* (Sakai *et al.*, 1982) and the North American species *H. rufescens* have a much narrower and longer acrosome, nucleus and nuclear anterior fossa than that in *H. varia*. The spermatozoon of *H. varia* also bears close resemblance to that of the trochoid gastropod *Tricolia capensis* described by Hodgson and Foster (1992).

A distinct anterior zone within the acrosomal vesicle of many haliotid spermatozoa have been reported; *H. discus* (Sakai *et al.*, 1982), *H. diversicolor supertexta* (Gwo *et al.*, 1997) and *H. laevigata* (Healy *et al.*, 1998). But no such differentiation in the acrosomal contents was discernible in the acrosomal vesicle of *H. varia* in the present study. Similar observation was made in *H. midae* by Hodgson and Foster (1992). In *H. varia*, the midpiece of the spermatozoon was comprised of four large mitochondria arranged closely. But the midpiece of the spermatozoa of *H. diversicolor supertexta* (Liu *et al.*, 1987) and *H. laevigata* (Healy *et al.*, 1998) contained five such mitochondria. So there are species differences among haliotids in the number of mitochondria in the spermatozoon midpiece.

Oogenesis in molluscs can be divided into a generative (proliferative) and vegetative (growth) phase (Anderson, 1974). During the proliferative phase the number of oogonial cells increases in the germinal zone by mitotic multiplication. In the vegetative phase of development, oocyte growth, principally due to vitellogenesis, is very considerable and requires substantial uptake and synthesis of nutritive materials (Pipe, 1987).

The germinal cells in the ovary of *H. varia* are cuboidal with a darkly stained nucleus. They are distributed in the germinal zone along the inner wall of the ovary and along the ovarian trabeculae. The cuboidal nucleus contains nucleolus and measures around 6 µm in length. As the germinal cells enlarge they become oval or round oogonial cells. The oogonial cells of *H. varia* are similar to the description given for *Mytilus edulis* by Pipe (1987). The secondary oogonia had a diameter of 11 µm and were dominated by a large ovoid nucleus with a diameter of 9 µm. The nucleus of the oogonial cells contained less condensed chromatin material scattered in the cytoplasm. Numerous ribosomes, mitochondria and lipid inclusions were discernible in the cytoplasm of oogonial cells. Similarly some irregularly shaped lipid inclusions along with the ribosomes and mitochondria were reported in the oogonial cell of sea urchin by Milloning *et al.* (1968). The oogonial cells of *H. varia* are found to be lined up within or in the close vicinity of the germinal epithelium.

The primary oogonia divide to form secondary oogonia, which enter into the first meiotic division and become primary oocytes. The most prominent feature of the young undifferentiated oocytes is the large nucleus (germinal vescicle), which may occupy more than half of the diameter of the cell. One of the first indications that an oogonium is differentiating into an oocyte is the appearance of the characteristic pore in the nuclear membrane (Verhey and Moyer, 1967). The fine structure of these pores was not discernible during the present study. Afzelius (1955) reported that these holes appear to be traversed by a cylinder, which extends with one end into the cytoplasm and other end into the nucleus. Their function may be related to transporting materials across the nuclear membrane and they exist throughout the primary stage.

In the present study, electron dense nucleolar material was observed to diffuse into the cytoplasm through the nuclear pores in the primary oocytes. The electron dense granular materials were seen near the outer nuclear membrane. The cytoplasm of the oocytes becomes strongly basophilic indicating considerable synthesis of protein. Many workers have reported that RNA materials are transported from the nucleus to the cytoplasm prior to the onset of vitellogenesis. Gowden (1961) suggested that growing oocytes of Chiton exhibited two phases of synthetic activity; the first phase was chiefly concerned with RNA production and second phase with protein yolk synthesis. Adiyodi and Subramoniam (1983) reported that such nuclear emissions are not uncommon in the oocytes of growing oocytes of various animal groups and pointed to the transfer of nucleolar materials to the ooplasm as a prelude to protein synthesis. Similarly, Minganti (1950) reported that the basophilia increased during the development of the oocytes of the gastropod *Lymnaea*.

Yolk is the nutritive material, often accumulated in substantial quantities in the ooplasm to meet the requirements of embryonic development independent of the maternal organism. Vitellogenesis is the process by which yolk, complete in all its organic and inorganic components, is built up within the oocyte. Proteins and lipids constitute the major part of the organic reserves of the yolk. Vitellogenesis in several different animals has been shown to be both auto and /or heterosynthetic (Droller and Roth, 1966; Dumont and Anderson, 1967). Autosynthetic vitellogenesis means the yolk is produced within the oocyte and no extra oocytic yolk material is incorporated. In heterosynthetic vitellogenesis, yolk is formed in different tissues other than the oocyte and it is then transported and incorporated by the oocyte.

In *H. varia*, the primary oocytes entered the vitellogenic phase at a diameter of 50 µm and because of that the basophilia of the ooplasm considerably reduced. When the vitellogenesis starts, the RNA synthesis and transport from the nucleus to the cytoplasm is considerably decreased. Similar observations have been reported in Chitons by Swift *et al.* (1956) that in the successive stages of oocyte growth, cytoplasmic basophilia decreased suggesting that either RNA synthesis had ceased entirely or that the rate of its synthesis was not commensurate with the oocyte's growth in volume. RNA synthesis appeared to be chiefly restricted to the first stage of oocyte growth prior to the protein yolk synthesis in *Chiton tuberculatum* (Swift *et al.*, 1956).

The procees of vitellogenesis in different bivalves and gastropod species are described by various workers (Selwood, 1968; Starke, 1971; Pipe 1987; Bottke, 1977; Rigby, 1979). In the present investigation using electron microscopy, the vitellogenesis in *H. varia* is found to be chiefly autosynthetic. There are many reasons to confirm the autosynthetic nature of vitellogenesis in the oocyte of *H. varia*. That will be discussed in the following section.

The occurrence of pinocytotic vesicles along the plasma membrane of the oocyte is taken as an important criterion for exogenous vitellogenesis, as it indicates the uptake of macromolecules (Jong-Brink *et al.*, 1983). In molluscs, morphological evidence for uptake of macromolecules is presented for a number of species (Selwood, 1968; Starke, 1971; Hill and Bowen, 1976). Incorporation of ferritin macromolecules directly from the blood in pinocytotic vesicles has been demonstrated in the oocytes of *Lymnaea stagnalis* and *Planorbarius corneus* (Bottke and Sinha, 1979, Bottke *et al.*, 1982). The lack of micropinocytotic vesicles on the oolemma has

been the sole evidence that the extra oocytic cells are not involved in the fabrication of yolk components in *H. varia*.

In molluscs, vitellogenesis is often associated with the Golgi apparatus, which concentrates the substances used in the formation of either proteid or fatty yolk or both (Worley, 1944). Yolk is suspected to arise from the Golgi apparatus in the Opisthobranch gastropod *Aplysia* (Bolognari, 1960) and *Naranax* (Worley and Worley, 1943). Young and DeMartini (1970) reported that the Golgi apparatus might be responsible for the accumulation of yolk in the oocytes of *H. rufescens*. Present ultrastrucure study of the ovary of *H. varia* revealed that both Golgi apparatus and rough endoplasmic reticulum are in close association with both proteid and lipid yolk materials. Concentric layers of rough and smooth endoplasmic reticulum encircling protein and lipid yolk molecules could be seen in the vicinity of the nucleus of early vitellogenic oocytes. Bolognari (1960) has convincingly demonstrated that protein yolk synthesis in the oocytes of two molluscan species occurs within the Golgi zones and that these Golgi zones are composed of concentric lamellae, which contain dark granules of about 300 A° diameter. Durfort (1976) considered the formation of concentric lamellae to be a means of increasing the area of protein synthesis.

Various cell organelles, viz. the endoplasmic reticulum, Golgi apparatus, mitochondria, autophagous vacuoles and multivesicular bodies have been assumed to be involved in the formation of yolk granules in molluscan oocytes (Raven, 1961; Srivastava, 1965; Huebner and Anderson, 1976). It is apparently the combined activity of a number of these organelles that leads to the formation of the complex proteinaceous yolk granules. Various membrane specializations comprising of rough and smooth endoplasmic reticulum could be seen in the developing oocyte of *H. varia*.

The lamellar structures observed include concentric lamellae and annullate lamellae. The concentric membrane specializations encompassing several granules could be seen in the developing oocyte. This may be identical to the Balbiani' s vitelline body, found in the oocytes of many molluscs, which play a role in vitellogenesis (Raverberri, 1966; Anderson, 1969; Taylor and Anderson, 1969). These Balbiani's vitelline body, which occurs in developing oocytes of a wide spectrum of species throughout the animal kingdom (Guraya, 1979) was observed in the vitellogenic oocytes of *Mytilus edulis* (Pipe, 1987). The presence of these cell organelles and membrane specializations in close proximity of the yolk granule reiterates the autosynthetic nature of vitellogenesis in *H. varia*.

The third point, which provides light to the autosynthetic nature of vitellogenesis in *H. varia* is the visible transport of electron dense nuclear material through the nuclear pores to the cytoplasm of the oocyte. These condensed materials are reported as RNA, which is synthesized in the nucleolus of the developing oocyte. Adiyodi and Subramoniam (1983) pointed to these nuclear emissions as a prelude to protein yolk synthesis.

In addition to the autosynthetic process, the possibility of heterosynthetic accumulation of yolk in *H. varia* may not be ruled out. The absence of pinocytotic vesicles along the oolemma does not mean that transport of materials from extra oocytic cells are not at all taking place during the vitellogenic phase. The mechanism of transport of large molecules themselves through membranes is described in many species. Selman and Arnold (1977) suggests that yolk precursors may be synthesized by the rough endoplasmic reticulum in the syncytial cytoplasm and the passage of these yolk precursors through the syncytial plasma membrane and

oolemma by this mechanism would go undetected in electronmicrographs. Similarly, Rothman (1975) argues that the kinetics of enzyme secretion in several different systems can best be explained by the transport of large molecules themselves through membranes in addition to the mass transport as vesicles. Ferritin is reported as an important component of yolk in gastropods (Fioroni and Schmekel, 1976) and this protein is derived probably from the digestive gland (Johnson *et al.*, 1962; Bottke, 1977). So more detailed biochemical as well as histochemical study is needed to confirm the heterosynthetic nature of vitellogenesis in *H. varia*.

Both protein yolk and lipid yolk are present in the mature oocytes of *H. varia*. Raven (1961, 1972) distinguished two types of yolk in the molluscan oocytes, proteinaceous yolk and lipid yolk. The first is contained in membrane bound platelets or granules. Lipid yolk is stored in droplets or globules lacking a limiting membrane. Similar types are found in the mature oocytes of *H. varia*.

The vitellogenic oocyte of *H. varia* ia surrounded by a vitelline coat consisting of a layer of about 0.5 μm thick, which is formed by electron dense amorphous material. The tips of the microvilli protrude slightly beyond the outer surface of this layer. Its conspicuously smooth inner surface is separated by a definite perivitelline space from the cytoplasmic membrane. Similar observations were made in the mature oocyte of *Mytilus edulis* by Pipe (1987). It is concluded that the primary egg membrane or the vitelline membrane in molluscs is formed by the oocyte itself (Raven, 1961; Huebner and Anderson, 1976). Novikoff and Holtzman (1976) reports that the vitelline layer, made up of protein and polysaccharide, is formed by the Golgi apparatus, which buds off small vesicles containing glycocalyx material that is deposited at the outer surface of the cell by exocytosis.

Cytoplasmic inclusions such as cortical granules, lipid droplets, mitochondria and yolk granules constituted intrinsic components of the mature oocyte of *H. varia*. The cortical granules are found to be arranged beneath the oolemma at the cortex of the oocyte. They were round to oval membrane bound bodies and 0.3 μm in diameter. They are not arranged as a continuous layer along the cortex of the oocyte in contrast to that found in *Mytilus edulis* (Pipe, 1987). Similarly, the cortical granules of *Placopecten* oocytes do not form a well-defined layer near the oolemma (Desilets *et al.*, 1995). Very little is known about the formation of cortical granules. Takashima (1960) suggested that the mitochondria have a close relationship to the formation of cortical granules. Anderson (1968) suggested that in *Arabacia punctulata* the Golgi complex plays a major role in the production of cortical granules.

Oocyte degeneration and resorption in molluscs may be brought about by a variety of natural environmental conditions such as extremes of temperature, desiccation or low levels of nutrition (Joosse *et al.*, 1968; Lucas, 1971; Jong-Brink, 1973; Bayne *et al.*, 1978). Alternatively, it may result from exposure to environmental contamination by pollutants (Lowe and Pipe, 1986). The result of oocyte degeneration and resorption is the recycling of nutrients to meet the energy requirements of basal metabolism (Herlin-Houtteville and Lubert, 1975).

In *H. varia*, during oocyte degeneration, the oocytes become globular, and the vitelline membrane is lifted off from the oolemma. The yolk granules are surrounded by autophagic vacuoles. The number of lipid droplets and lysosomes increase sharply in the ovarian trabeculae. Genital blood vessels also discernible near the degenerating oocyte. The vitelline membrane degenerates followed by the break

down of the cytoplasmic membrane of the oocyte releasing the contents to the ovarian lumen.

Oocyte degeneration occurs in different stages of oocyte development in different species. In *Viviparous viviparous*, oocyte degenerate in all stages of development (Griffond, 1977). The study of histological sections of the gonad of fresh water snail *Biomphalaria glabrata* shows that only 'ripe' oocytes ie., oocytes surrounded by a follicular cavity, degenerate. But in *H. varia*, oocyte degeneration was observed in early vitellogenic, late vitellogenic and mature oocytes.

In the present study, spawning season and annual reproductive cycle of *H. varia* were determined from gonad index, as has been mainly used in several species (Ino and Harada, 1961; Boolootian *et al.*, 1962; Newman, 1967). It has been done in *H. varia* at Phuket, Andaman Sea Coast of Thailand by Bussarawit *et al.* (1990). Percentage occurrence of male and female maturity stages over the sampling period also has been taken in to consideration in determining the annual reproductive cycle of *H. varia* in the present study.

Bussarawit *et al.* (1990) suggests that in *H. varia* at Phuket, as a rapid post spawning recovery was observed during their study, there was a greater gametogenic activity in tropical than the temperate species. For *H. varia*, in the present study, annual reproductive cycle was studied for a period of 15 months. Almost all the stages of gametogenic activity were found throughout the period of sampling. Similar observations were made by several authors. Wells and Keising (1989) reported that throughout the 2 year collection period, most abalones (*H. roei*) were in the recovery II stage except during the height of spawning season. For *H. varia*, in both the stations, early maturing and late maturing stages were observed in all the months over the

sampling period. Ripe male gonads were obtained in most of the months over the sampling period. But ripe female gonads were restricted to the breeding season. Similarly, ripe male specimens were present in the population of *H. roei* throughout the 2 years studied by Wells and Keising (1989) but in general their numbers were very low. Rothschild (1935) found in *Teritella communis* that eggs matured only periodically in the population but that the testes had mature sperms throughout the reproductive cycle. A similar situation was found by Webber and Giese (1969) in *H. cracherodii.*

During the spawning season, the animals quickly moved from maturing stage to ripe or partially spawned or spent stage. For the Tuticorin population, gonad index was highest in December to February months. Completion of spawning was observed in May and immediately after spawning, most of the gonads were in a state of recovery. From July to November, the GSI was low and the lowest value observed was in August. More number of ripe animals were present when the GSI value of the abalones were higher ie., from October to February.

At Mandapam, the gonad index showed highest value in December and remained higher from November to January. The percentages of ripe and partially spawned animals during these periods were higher compared to the other months. This indicates spawning during this period. So from the above results, it is evident that *H. varia* of Tuticorin and Mandapam stations follow a more or less distinct pattern of annual reproductive cycle with a short breeding period extending from December to March in Tuticorin and November to February in Mandapam. The differences in the breeding season in two populations have been reported by many authors. Wood and Buxton (1996) reported that slight differences in the duration of spawning period

between close populations are not unusual among haliotid species. Newman (1967) found sufficient difference in the timing and intensity of spawning in *H. midae* at three sites. Muller (1984) found a shift in the breeding season in *H. sporadica* from two stations only 12 Km apart.

Shepherd and Laws (1974) emphasize the impact of local environmental conditions in the regulation of reproductive periodicity. In Indian coasts, intensities of breeding differ in accordance with the Southwest and Northeast monsoon (Pillai and Nair, 1971; Varadarajan and Subramoniam, 1982a) thus showing distinct peaks in the reproductive cycle. For *H. varia*, the breeding cycle coincides with the end of Northeast monsoon.

Unlike *H. varia*, many other abalone species are capable of spawning throughout the year. Booloottian *et al.* (1962) reported that *H. rufescence* may spawn at all times of the year, but other authors (Carlisle, 1962; Cox, 1962) believed that actual spawning occurs only over a more restricted period. In South Australia, *H. roei* was found to be capable of spawning throughout the year (Shepherd and Laws, 1974). But Webber and Giese (1969) reported that *H. cracherodii* spawned only during a 6-week period during the year in August and September.

In marine molluscs, exogenous factors that initiate and control gametogenesis are not well known. Fretter and Graham (1964) discussed the effect of environment on the breeding in molluscs, noting that maturation of gametes is initiated by annual temperature fluctuations, but a combination of other factors may trigger spawning. The ability of an organism to reproduce depends upon adequate supply of energy to meet maintenance as well as reproductive requirements during its gametogenic cycle. The availability of adequate energy depends on the availability of food and the

metabolic rate of the animal, which to a certain extent is influenced by environmental temperatures (Barber and Blake, 1983). Reproductive cycles are often correlated with sea temperatures (Orton, 1920; Giese, 1959).

In the present study, the temperature values fluctuated much over the study period and lower values were observed during the Northeast monsoon period and the post monsoon period. The seasonal low temperature values were found to coincide with the breeding season of *H. varia* both at Tuticorin and Mandapam stations. During the active gametogenic period and recovery period the temperature value observed were found to be higher. Similarly, in the graper clam *Tresus capax*, Machell and DeMartini (1971) reported that the active phase was associated with the period in which highest temperature and salinity measurements were obtained. Spawning occurred during seasonal low values. As many other factors such as feeding habits and density of abalones were not studied during the present investigation, it cannot be concluded that temperature is the only exogenous factor influencing the annual reproductive cycle of *H. varia*. No significant difference in salinity values was observed during the present sampling period. Webber and Giese (1969) report that no evidence exists for a possible role of salinity directly regulating gametogenic activity in archaeogastropods. Similarly, no significant correlation was obtained in the pH and dissolved oxygen values with GSI over the entire study period of the reproductive biology of *H. varia*.

Chapter III

BIOCHEMICAL CHANGES IN RELATION TO GONAD MATURATION

1. Introduction
2. Materials and Methods
3. Results
4. Discussion

INTRODUCTION

Most marine invertebrates have annual reproductive cycles. The variation in the biochemical composition of the whole body as well as individual organ systems during different stages of gonad development and maturation have been taken into consideration in determining these annual reproductive cycles (Giese, 1959, 1967, 1969; Giese and Pearse, 1974). For the purpose of biochemical characterization from this point of view, the body of a mollusc, therefore, is divided into 'body components', which are parts that can be conveniently separated from one another by dissection and are of sufficient size to permit biochemical analysis. The body of a gastropod may be divided in this way into shell, head, foot, mantle, viscera, gonad, digestive gland and blood.

In the determination of the body component indices it is apparent that some indices such as gonad index, hepatic index and foot index, vary with the reproductive season. Sometimes organs other than gonads vary with the reproductive season. Gonadal maturity marks a change in the growth pattern of many molluscs, resulting from the 'reproductive drain' of materials meant for somatic growth to the gonads. Proximate composition analysis of an animal during gonadal development can also disclose the energy banks of various nutrients in the body and trace out the pathway through which they are mobilized to the gonad.

To study the physiology of an animal, the biochemical constitution of each of the component organs of the body has been found to be more informative than an analysis of the entire body. The size of mature gonad is large, compared to the total

body mass in marine invertebrates that release their gametes to the environment. A major problem in such broadcast fertilizer reproductive pattern is the nature of the energy source for gamete production. Specifically, during the reproductive cycle, nutritive energy is stored when gonads are not active and mobilized during periods of rapid gonad growth (Giese, 1966 and 1969). For example, in oysters and clams nutrient storage occurs in the gonad itself and the metabolite is glycogen (Masumoto *et al.*, 1934; Giese *et al.* 1967).

Investigations on the biochemical changes during the reproductive cycle of different classes of mollusca have been made by many researchers. Variations in the biochemical composition of different tissues of molluscs during prespawning, spawning and post spawning periods have been observed with special reference to moisture, protein, lipid, carbohydrates and ash. Such variation in Amphineurans, has been studied in detail by Giese and Araki (1962) while the monthly protein level in body components of amphineurean *Katharina* has been studied by Giese and Hart (1967). On the same species, fractionation of lipid into neutral lipid (unsaturated fatty acids, triglycerides and sterols) and polar lipids (phospholipids) was investigated by Lawrence and Giese (1969). The lipid levels during an entire reproductive cycle have also been studied in *Katharina*. Variability in some of the body component indices with the course of the reproductive season has been documented (Giese *et al*, 1959; Giese and Araki, 1952; Giese and Hart, 1967, Tucker and Giese, 1962).

In many lamellibranchs, it is difficult to separate the body components from one another. In *Mytilus* and *Modiolus*, the gonad and mantle form a unit, the gonad proliferating into the mantle. In spite of that, considerable information exists on the biochemistry of bivalves, especially on the oysters. Much of the works on oysters

have been reviewed by Galtsoff (1964). Seasonal variation of biochemical levels in the oyster *Ostrea edulis* has been reported by Russell (1923) and Couteaux-Bargeton (1947). In *Crassostrea virginica*, the carbohydrate level is very high in the immature animal and falls precipitously as gametes are formed in the gravid animal (Lee and Pepper, 1956).

Some of the data for the Japanese pearl oyster *Pinctada martensii* are given by Tanaka and Hatano (1952). Seasonal changes in the biochemical composition of the bivalve *Donax trunculus* have been studied by Ansell *et al.* (1973). Ansell (1974) has investigated on the seasonal changes in biochemical composition of the bivalve *Abra abra*. Chemical composition of Taiwanese oysters and clams were studied by Jeng *et al.* (1979). More information has been given by Shafee (1981) about the relation of biochemical composition and reproductive cycle in the black scallop *Chlamys varia*.

In contrast to the voluminous studies on the oysters and other lamellibranchs, biochemical studies on the gastropods is limited. Some of the earliest work on gastropods was done by Barry and Munday (1959) on the tissues of *Patella*. The gonad indices in the abalones *Haliotis cracherodii* and *H. rufescens* have been studied in detail (Boolootian *et al.*, 1962). High glycogen levels have been reported in the snail *Acanthina* (Giese 1966b). Seasonal variation in biochemical components has been studied in *Patella vulgata* (Blackmore, 1969) in and *Turbo sarmations* (McLachlan and Lambard, 1980). Seasonal changes in condition factors and in the C:N ratio of the foot of the ormer *Haliotis tuberculata* has been studied by Hayashi (1983).

Considerable works have been reported during the last decade on the biochemical study of gastropod molluscs including *Haliotis* spp. from various countries. Digestive enzymes of the saltmarsh periwinkle *Littorina irrorata* were studied by Baerlocher *et al.* (1989). Oates *et al.* (1990) have used open tubular liquid chromatography to analyze the amino acid content of individual neurons of the snail *Helix aspersa*. Jimenez-Arce (1993) studied the chemical composition in the different sizes and sexes of *Strombus gracilior* from Panama Beach, Costa Rica. Effects of algal diets on the growth and biochemical composition of *Haliotis discus hannai* were given by Mercer *et al.* (1993). Watanabe *et al* (1993) studied the changes in the contents of extractive components in the disk abalone fed with marine algae and starved. Proximate composition of the South African abalone, *Haliotis midae* has been studied by Knauer *et al.* (1994).

Along the Indian coast, chemical studies have been made on the Indian oyster *Crassostrea gryphoides* (Durve and Bal, 1961). Wood boring molluscs have been subjects of various biochemical investigations in India eg. *Martesia striata* (Nagabhushanam, 1961, Sreenivasan, 1963; Sreenivasan and Krishnaswamy, 1964). The biochemical composition of four invertebrates including *Donax* sp. taken from sandy beaches of Cochin and Shertallai has been studied by Ansell *et al.* (1973). Nagabhushanam and Dheshmukh (1974) studied the seasonal changes in chemical composition in the estuarine clam *Meretrix meretrix*. Seasonal changes in meat weight and biochemical composition have been reported in the black clam *Villorita cyprinoids* (Ansari *et al.*, 1981). The other works on biochemical composition in Indian molluscs are on *Katelysia marmorata* (Joshi and Bal, 1965), *Nausitora hedleyi* (Saraswathy and

Nair, 1969), *Meretrix casta* (Salih, 1979; Balasubrahmanyan and Natarajan, 1980) as well as in *Donax cuneatus* (Victor, 1984)

In recent years studies on the biochemical composition of some bivalves as well as gastropods have been reported from Indian coast. Sarvaiya (1989) studied the shell and meat composition of some pelecypods and gastropods from the Saurashtra coast. Studies on the bioactivity and chemical ecology of some opisthobranch molluscs have been carried out by Parulekar and Sirvoiker (1991). Jayabal (1994) studied the biochemical contents in the estuarine clam *Katelysia opima* from Vellar Estuary. Seasonal variations in the metal contents of *Suneta scripta* were studied by Pillai and Valsala (1995). Proximate biochemical composition and calorific potential of raft cultured green mussel *Perna virdis* were studied by Revonker and Parulekar (1995). Karthikeyan and Kumaraswamy (1998) studied the effect of cadmium concentrations on the biochemical composition of the estuarine clam *Meretrix casta* in different salinities. Pharmacological studies on the venom of the gastropod mollusc *Conus inscriptus* have been carried out by Singaravadivelan *et al.* (1998). George and Damodaran (1999) have studied on the variations in amino acid content in the intracellular fluid during the isosmotic regulation in the yellow clam *Suneta scripta.*

The basic metabolic levels of various biochemical components in prosobranchs are, very high lipid levels and low polysaccharide levels in the gonad and high polysaccharide and low lipid levels in muscular tissue, particularly in the foot. High gonad lipid levels (particularly in ovaries) have been described in *Haliotis* species (Albrecht, 1923; Giese, 1966; Webber, 1967). Ovary lipid apparently is the energy source for the egg yolk.

Carbohydrate (glycogen) levels are generally low in the gonad tissue of Prosobranchs while muscle tissue is relatively rich in carbohydrates (*Haliotis*: Giese 1966; Webber 1967). The possibility that glycogen in the muscle tissue of *H. cracherodii* is used for gonad growth during periods of active gametogenesis was described by Webber (1967). However, there is no direct evidence that glycogen is used as an energy source in the formation of gonad.

In the present investigation, biochemical composition of three tissues, *viz.*, foot, digestive gland and gonad of abalone, *Haliotis varia* has been studied. Five major parameters such as moisture, proteins, lipids, carbohydrates and ash have been estimated in relation to the maturation of gonad.

MATERIALS AND METHODS

1. COLLECTION OF SPECIMENS

Male and female specimens of *Haliotis varia* in different maturity stages of were collected from the intertidal region of Tuticorin harbour basin and transported to the Mandapam Regional Centre laboratory as mentioned in the Materials and Methods of Chapter II.

2. BIOCHEMICAL ANALYSIS

Moisture, total proteins, total carbohydrates, total lipids and ash contents in the foot, digestive gland and gonad tissues were estimated during different stages of gonad maturation of *H. varia*. After ascertaining the maturity stage of the gonad, foot, gonad and digestive gland were separated from the body. These three components were made upto about 75% of the soft part weight of the animal. All the tissue samples were accurately weighed to the nearest milligram in an electronic balance (Sartorius BP210D). When adequate amount of tissues were not available from a single specimen, tissues from different specimens belonging to the same stage of maturity were pooled for analysis.

After determining the moisture content, the dried samples were powdered in a mortar, transferred to labeled polythene bags and stored in a desiccator for further analyses. For all the estimations extra pure of 'Anala R' grade chemicals only were used.

The optical density of the colour developed for total proteins, carbohydrates and lipids were measured using a UV/VS spectrophotometer (GBC 911A), with the samples taken in silica cuvetts. Standard graphs were plotted with the concentration of

each biochemical parameter in different dilutions of the working standard solution in the X-axis and optical density in the Y-axis. The concentrations of different parameters in the samples were calculated (in mg%) by comparing the optical density (O.D.) obtained for the sample with the values in the standard graph and also using the following formula.

Concentration in mg /100 mg dry tissue:

$$\frac{\text{(O.D. of the sample-O.D. of the blank)}}{\text{(O.D. of the standard-O.D. of the blank)}} \times \text{concentration of standard} \times \frac{100}{\text{weight of the sample in mg}}$$

2. 1. MOISTURE CONTENT

The tissue samples were cleaned and the water adhering to them was removed using a blotting paper. The wet weights of the tissues were taken accurately and the samples were gradually dehydrated to constant weight in a hot air oven at 70 ºC. The moisture content was then calculated gravimetrically as the difference in the wet weight and the dry weight of the tissue and was expressed as percentage of wet weight.

2. 2. TOTAL PROTEINS

The Folin-Ciocalteu phenol method of Lowry *et al.* (1951) was adopted for the estimation of total proteins in the tissues.

Dry tissue samples (foot, digestive gland and gonad), each weighing 10 mg were thoroughly homogenized with 2 ml deproteinising agent (10% trichloro acetic acid) by keeping the tubes in ice. All the samples were centrifuged for 20 minutes at 3000 rpm. The supernatant obtained in the individual tubes was used for the

estimation of total carbohydrate. The protein precipitate in each tube was dissolved in 5 ml 1N NaOH. From this solution 0.1 ml was taken in triplicate and made up to 0.5 ml with double distilled water. To this 0.5 ml solution, freshly prepared 5 ml alkaline mixture (50 ml of 2% Na_2CO_3 in 0.1N NaOH + 1 ml of 0.5% $CuSO_4$ $5H_2O$ in 1% sodium potassium tarterate) was added and kept at room temperature for 10 minutes. After 10 minutes 0.5 ml of 1N Folin-Ciocalteu's reagent (diluted the 2N stock solution with double distilled water) was added and mixed rapidly.

A standard stock solution was prepared using bovine serum albumin crystals at a concentration of 25 mg /5 ml 1N NaOH. Different dilutions in the range of 0.25 to 2.5 mg/ml were prepared from this stock solution, and the alkaline mixture and Folin-Phenol reagent were added as in the case of tissue samples. A blank was prepared with 0.5 ml double distilled water and treated the same as above.

All the test tubes were kept for 30 minutes at room temperature and the optical density of the blue colour developed was measured against the blank at 660 nm.

2. 3. TOTAL CARBOHYDRATES

The phenol sulphuric acid method of Dubois *et al.* (1956) was followed to estimate the total carbohydrates in the samples.

The supernatant obtained during protein estimation procedure was used for the analysis. From the above supernatant, 0.1 ml was taken and made up to 1 ml with saturated solution of benzoic acid in double distilled water and to this solution, 1 ml of 5% phenol solution was added. 5 ml of concentrated sulphuric acid was added rapidly and carefully to each tube and mixed well using a cyclomixer.

A standard solution was prepared using D-glucose (concentration –20 mg/ 100 ml saturated solution of benzoic acid). Different dilutions of the working solution with

the concentration of glucose ranging from 10-100 μg/ml were prepared and the procedure adopted for the tissue was followed. A blank solution with 2 ml 5% phenol was prepared and the above procedure followed.

All the tubes were kept for 30 minutes at 30 °C and the optical density of the orange colour developed was measured at a wavelength of 490 nm.

2. 4. **TOTAL LIPIDS**

The total lipids were quantitatively determined by sulphophosphovannillin method of Barnes & Blackstock (1973).

About 10 mg of foot and gonad and 5 mg digestive gland samples were separately homogenized well in 2 ml of chloroform: methanol (2:1 v/v) and kept overnight at 4 °C for complete extraction. The mixture taken in glass stoppered centrifuge tubes were then centrifuged for 15 minutes at 3000 rpm and the clear supernatant containing all lipids was transferred to clean, dry glass tubes. 0.5 ml of this lipid extract of all the tissues were taken separately in clean glass tubes and dried *in vacuo* over silica gel in a desiccator. To each dried sample, 0.5 ml of concentrated sulphuric acid was added and shaken well. The tubes were then plugged with non-absorbent cotton wool and heated at 100 °C in a boiling water bath exactly for 10 minutes. The tubes were rapidly cooled to room temperature under running tap water. To 0.1 ml of this acid digest, 2.5 ml of phosphovanillin reagent was added and mixed well in a cyclomixer.

Stock solution was prepared afresh by dissolving 80 mg of cholesterol in 100 ml of chloroform: methanol (2:1 v/v) mixture (equivalent to 100 mg of total lipid in 100 ml (2:1 v/v) chloroform: methanol mixture). Working solutions of different concentrations

were prepared from the stock solution in the range 50-500 µg/0.5 ml and the procedure adopted for the tissue samples was followed. 0.5 ml of 2:1 v/v chloroform: methanol mixture was treated as blank.

All the tubes were kept at room temperature for 30 minutes. The intensity of the pinkish red colour developed was measured against blank at 520 nm.

2. 5. ASH CONTENT

A pre weighed amount of oven dried powdered tissue samples was ignited in a silica crucible for 5 hours at 600 °C in a muffle furnace, till all the organic matter was burnt out leaving no carbon residue. The ignited content was weighed and the difference in the weight gave the ash content of the tissue. The percentage of the ash content of the tissue was calculated as follows.

$$\text{Ash mg\%} = \frac{\text{Ash weight}}{\text{Dry weight of tissue}} \times 100$$

RESULTS

Concentrations of the metabolic components like moisture, protein, lipid, carbohydrates and ash showed significant variations in the various tissues of *Haliotis varia*, during the progress of gonad maturation. The analytical results of parameters in tissues like foot, liver and gonad during the various stages of maturation in male and female are given in tables 20 to 25 and graphically represented in figures 25 to 30.

1. FOOT

Moisture : In the female, moisture content gradually increased from 71.29% in stage I to 72.56% in stage III. The highest value observed was 73.33% in stage V. In male, water content ranged between 71.33% and 75.38%, with low value recorded in stage IV, and maximum, 75.66% in stage VI.

Protein: Protein values were higher in the muscular foot tissue than that in the other tissues, ranging from 65.84 to 76.39 mg% (stage VI). In female, protein value was highest in stage IV (74.55 mg%) and lowest in stage II (68.11 mg%). In male, the highest value observed was 75.35 mg% in stage II and lowest was 65.84 mg% in stage IV.

Lipid : Lipid content showed less variation in female foot tissue, with a value of 4.100 mg% in stage I, which gradually increased to 6.620 mg% in stage III. This gradually declined to 4.912 mg% in stage V. In male, the lipid value ranged between 2.627 mg% in stage V and 7.797 mg% in stage IV, which decreased to 2.640 mg% in stage VI.

Carbohydrate : The carbohydrate content in the foot tissue was found to be generally poor. In female, it increased from 0.98 mg% in stage II to 4.528 mg% in

stage IV, with the highest value in stage V (5.867 mg%). In male the carbohydrate content was found to be much variable with a lowest value of 0.273 mg% in stage I, which gradually increased to the highest value of 11.18 mg% in stage IV. In stage VI the value observed was low, 1.792 mg%.

Ash : In female, the ash content did not show any significant variation. The value exhibited a narrow range of 6.023 mg% in stage V to 6.835 mg% in stage I. In male, it was lowest in stage III (6.224 mg%) and highest in stage V (7.821 mg %). The ash value observed in stage VI was 6.632.

2. DIGESTIVE GLAND

Moisture : Low moisture content 61.54% in stage I, was recorded in female digestive gland tissue, which gradually increased to a maximum of 68.92% in stage IV. In male liver tissue, the water content was little higher than that in the female tissue with a minimum of 64.00% in stage IV and maximum of 74.29 % in stage II.

Protein : The protein content in the digestive gland was found to be generally poor. In female, maximum protein content was observed in stage II (60.58 mg%) and the minimum value of 48.67 was obtained in stage I. In male, it ranged between 49.36 mg% in stage IV and 55.41 in stage V. In stage VI, the value obtained was 50.41 mg%.

Lipid : Lipid values were generally high in digestive gland tissue. In female, minimum 12.863 mg% was in stage II and maximum of 17.412 mg% in stage V. In male, the lipid variation was more significant with the lowest observed in stage I (8.721 mg%) and highest in stage III (15.07 mg%). The value obtained was 8.260 mg% in stage VI.

Carbohydrate : In female, the carbohydrate values ranged between 9.567 mg% in stage V and 15.670 mg % in stage III. The male liver carbohydrate content was little lower than that in female with a minimum value of 6.885 mg % in stage V and maximum value of 11.268 mg% in stage III. The highest value of carbohydrate in liver tissue was obtained in the stage VI animal (13.527 mg%).

Ash : The ash content was much higher and highly variable in the female digestive gland tissue. It ranged between 9.825 mg% in stage II and 14.625 mg% in stage I. The male liver ash content was high in all the stages. In stage II it was 12.634 mg% and the maximum value was in stage V with a value of 16.792 mg%. In stage VI, the value observed was 12.036 mg%.

3. GONAD

Moisture: In female, the moisture content gradually increased from 61.54% in stage I to 71.43% in stage V. It was much higher in the testis than that in the ovary with a minimum value of 70.94% in stage IV and maximum value of 78.74% in stage V. The value observed was 74.23% in stage VI.

Protein: The protein content of the ovary fluctuated between 54.06 mg% in stage IV and 58.79 in stage II. In the testis, the value ranged from 54.92 mg% in stage IV to 64.75 mg% in stage II. The stage VI gonad obtained 60.82 mg% of protein.

Lipid: The ovary lipid value was much higher than that in the testis. The highest lipid content observed was in the stage III ovary (26.492 mg%). The lowest value obtained was in the stage V (12.604mg%). In the testis, the lipid content varied between 5.250 mg% in stage V and 10.110 mg% in stage IV. The stage VI gonad had a lipid value of 6.148 mg%.

Carbohydrate: In the ovary, the carbohydrate value ranged between 1.825 mg% in stage V and 7.734 mg% in stage II. In the testis, the value fluctuated between 1.736 mg% in stage IV and 4.229 mg% in stage III. The gonad in stage VI contained 4.877 mg% carbohydrate.

Ash : The ash content of the ovary fluctuated in a narrow range between 6.081 mg% in stage V and 7.579 mg% in stage III. In the testis, the minimum value observed was in stage I (7.635 mg%) and the maximum value obtained was in stage V (12.060 mg%). In the indeterminate gonad (stage VI) ash content was found to be 6.980 mg%.

4. Statistical analysis

The analysis of variance (ANOVA) was carried out for each biochemical parameter in foot, digestive gland and gonad to test significant changes i) between different tissues at various stages of maturity and ii) between different stages of maturity in various tissues. The results indicated significant variation of all parameters between tissues at 5% level. As the interaction between tissues and stages was found to be significant at 5% level, the individual parameter in a particular tissue was subjected to one-way ANOVA to test the variation between different stages. The results showed that except moisture and ash, all the other parameters were found to vary significantly between stages at 5% level. The calculated F values for different parameters between stages are shown in the tables 20 to 25.

Table 20. Variations in the biochemical composition in the foot of female *H. varia* (dry weight basis)

Maturity Stages	Moisture (%)	Total proteins (mg%)	Total lipids (mg%)	Total carbohydrates (mg%)	Ash (mg%)
Stage I	71.29 ± 2.45	73.57 ± 3.26	4.100 ± 0.831	1.974 ± 0.702	6.835 ± 1.245
Stage II	72.13 ± 3.64	68.11 ± 2.85	4.860 ± 0.516	0.981 ± 0.522	6.632 ± 0.852
Stage III	72.56 ± 3.42	70.35 ± 3.97	6.621 ± 2.890	4.526 ± 1.701	6.575 ± 0.853
Stage IV	70.09 ± 4.25	74.55 ± 7.40	5.373 ± 0.806	5.867 ± 0.851	6.256 ± 0.426
Stage V	73.33 ± 2.30	72.47 ± 2.48	4.912 ± 0.916	2.257 ± 0.199	6.023 ± 0.872
Stage VI	75.66 ± 3.26	76.39 ± 5.25	2.641 ± 0.612	1.792 ± 0.421	6.632 ± 0.654
F value $_{(5, 30)}$	6.305*	4.442*	9.870*	45.099*	3.729*

*: Significant at1% level (P<0.01)

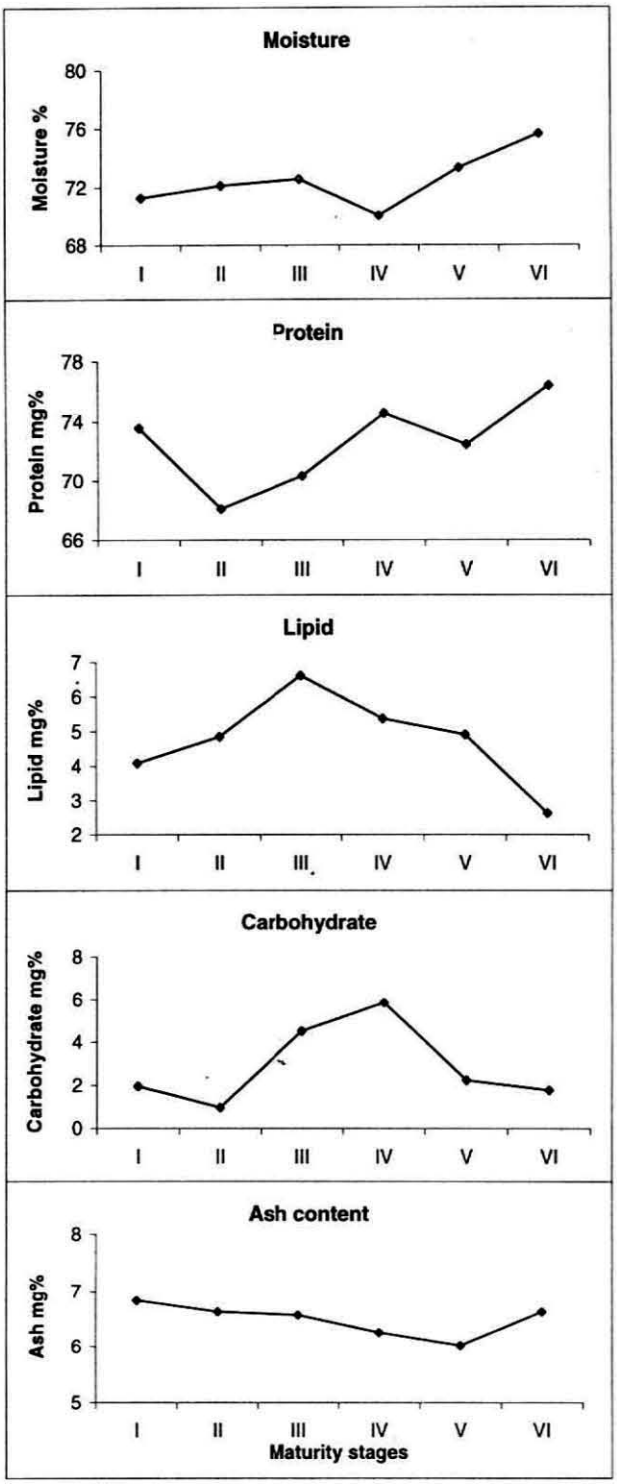


Fig. 25. Trends in the variation of biochemical components in the foot tissue of female *H. varia*

Table 21. Variations in the biochemical composition in the digestive gland of female *H. varia* (dry weight basis)

Maturity Stages	Moisture (%)	Total proteins (mg%)	Total lipids (mg%)	Total carbohydrates (mg%)	Ash (mg%)
Stage I	61.54 ± 2.29	48.67 ± 4.95	15.473 ± 1.453	13.337 ± 2.056	14.625 ± 0.525
Stage II	63.25 ± 1.96	60.58 ± 2.86	12.863 ± 0.372	11.020 ± 2.424	9.825 ± 0.373
Stage III	64.44 ± 2.16	53.30 ± 7.73	16.514 ± 2.890	15.670 ± 5.736	12.525 ± 0.375
Stage IV	68.92 ± 1.40	53.81 ± 11.04	16.030 ± 1.149	11.640 ± 1.052	13.865 ± 0.514
Stage V	59.55 ± 2.98	52.36 ± 2.11	17.412 ± 1.667	9.567 ± 0.689	14.256 ± 1.326
Stage VI	66.66 ± 1.67	50.41 ± 1.34	8.260 ± 2.066	13.527 ± 2.847	12.036 ± 0.819
F value $_{(5, 30)}$	15.176*	2.837**	22.213*	3.187**	3.729*

*: Significant at1% level (P<0.01), **: significant at 5% level (P<0.05)

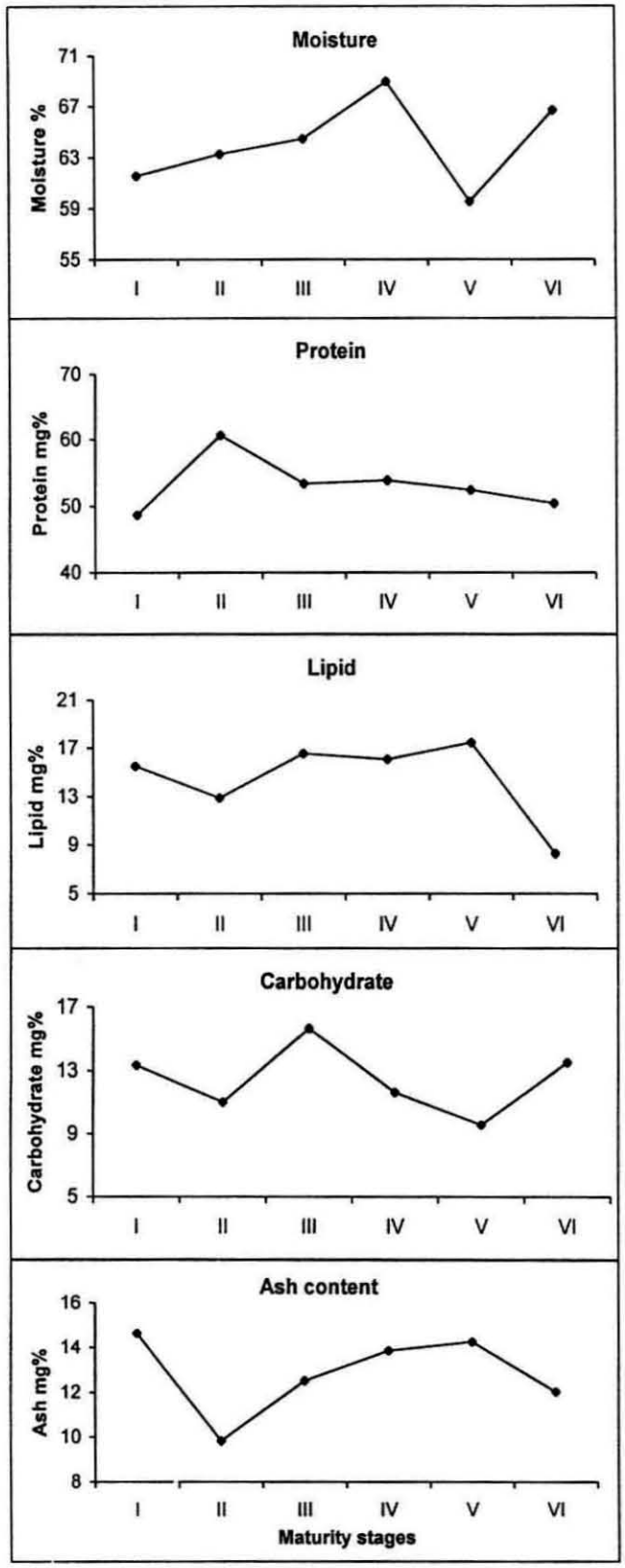


Fig. 26. Trends in the variation of biochemical components in the digestive gland tissue of female *H. varia*

Table 22. Variations in the biochemical composition in the gonad of female *H. varia* (dry weight basis)

Maturity Stages	Moisture (%)	Total proteins (mg%)	Total lipids (mg%)	Total carbohydrates (mg%)	Ash (mg%)
Stage I	61.54 ± 2.25	62.42 ± 5.48	16.364 ± 1.270	5.502 ± 0.560	6.124 ± 0.327
Stage II	62.46 ± 1.46	56.79 ± 4.69	20.005 ± 0.890	7.734 ± 0.392	6.253 ± 0.319
Stage III	63.35 ± 2.58	55.45 ± 4.97	26.492 ± 4.640	4.768 ± 0.544	7.579 ± 0.362
Stage IV	66.82 ± 1.64	54.06 ± 6.63	18.355 ± 0.926	2.153 ± 0.603	6.356 ± 1.198
Stage V	71.43 ± 2.09	57.56 ± 3.79	12.604 ± 0.778	1.826 ± 0.434	6.081 ± 0.235
Stage VI	74.23 ± 1.05	60.82 ± 3.56	6.148 ± 0.935	4.877 ± 0.695	6.980 ± 0.261
F value $_{(5, 30)}$	51.726*	2.472	76.632*	121.282*	25.380*

*: Significant at1% level (P<0.01)

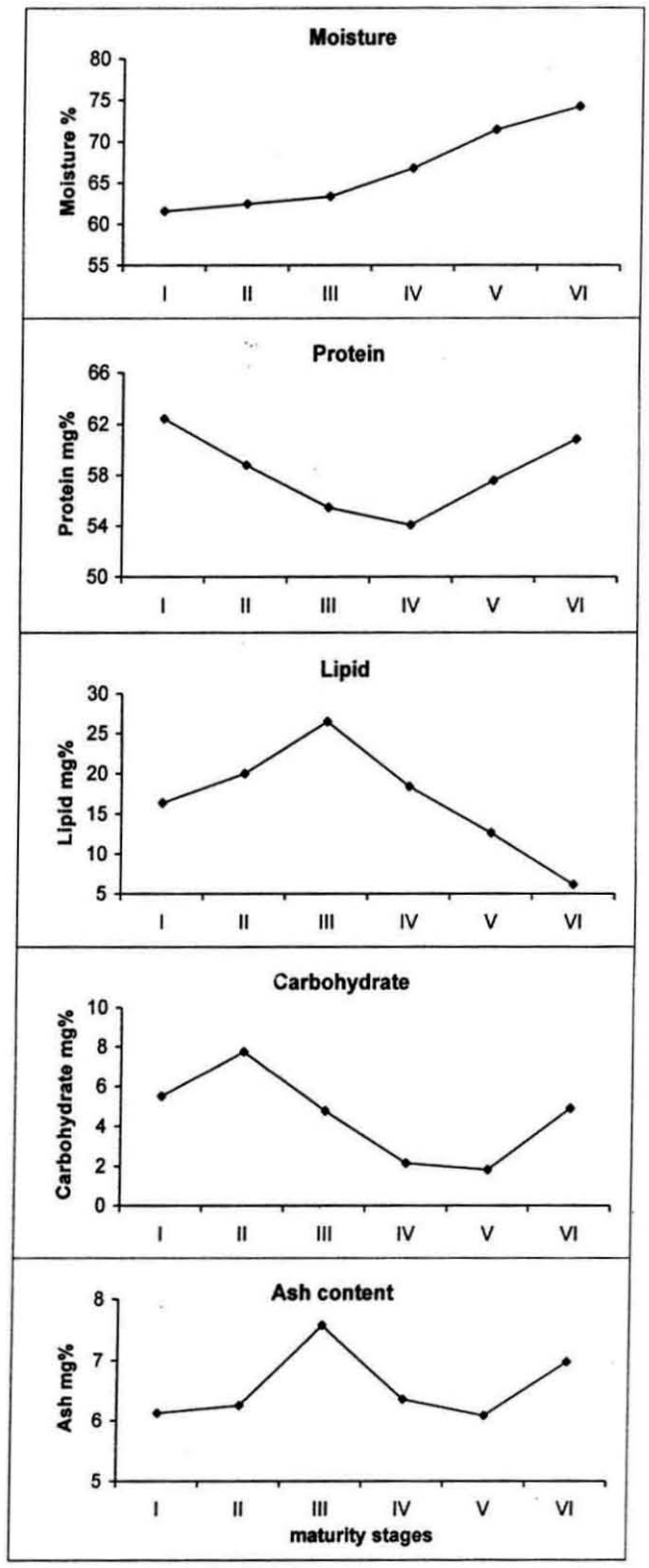


Fig. 27. Trends in the variation of biochemical components in the gonad tissue of female *H. varia*

Table 23. Variations in the biochemical composition in the foot of male *H. varia* (dry weight basis)

Maturity Stages	Moisture (%)	Total proteins (mg%)	Total lipids (mg%)	Total carbohydrates (mg%)	Ash (mg%)
Stage I	72.34 ± 3.15	74.50 ± 4.29	5.248 ± 0.102	0.273 ± 0.154	6.757 ± 0.407
Stage II	75.36 ± 3.45	75.35 ± 3.12	3.349 ± 0.015	2.880 ± 0.512	6.522 ± 0.542
Stage III	75.38 ± 1.50	71.06 ± 3.67	5.200 ± 0.218	5.647 ± 1.256	6.224 ± 0.522
Stage IV	71.33 ± 1.85	65.84 ± 3.13	5.797 ± 1.211	11.180 ± 3.889	6.818 ± 1.430
Stage V	75.25 ± 2.85	69.13 ± 5.03	2.627 ± 0.098	4.178 ± 1.513	7.821 ± 1.484
Stage VI	75.66 ± 3.36	76.39 ± 3.73	2.640 ± 0.285	1.792 ± 0.416	6.632 ± 0.464
F value $_{(5, 30)}$	5.199*	6.649*	45.794*	103.718*	7.796*

*: Significant at1% level (P<0.01)

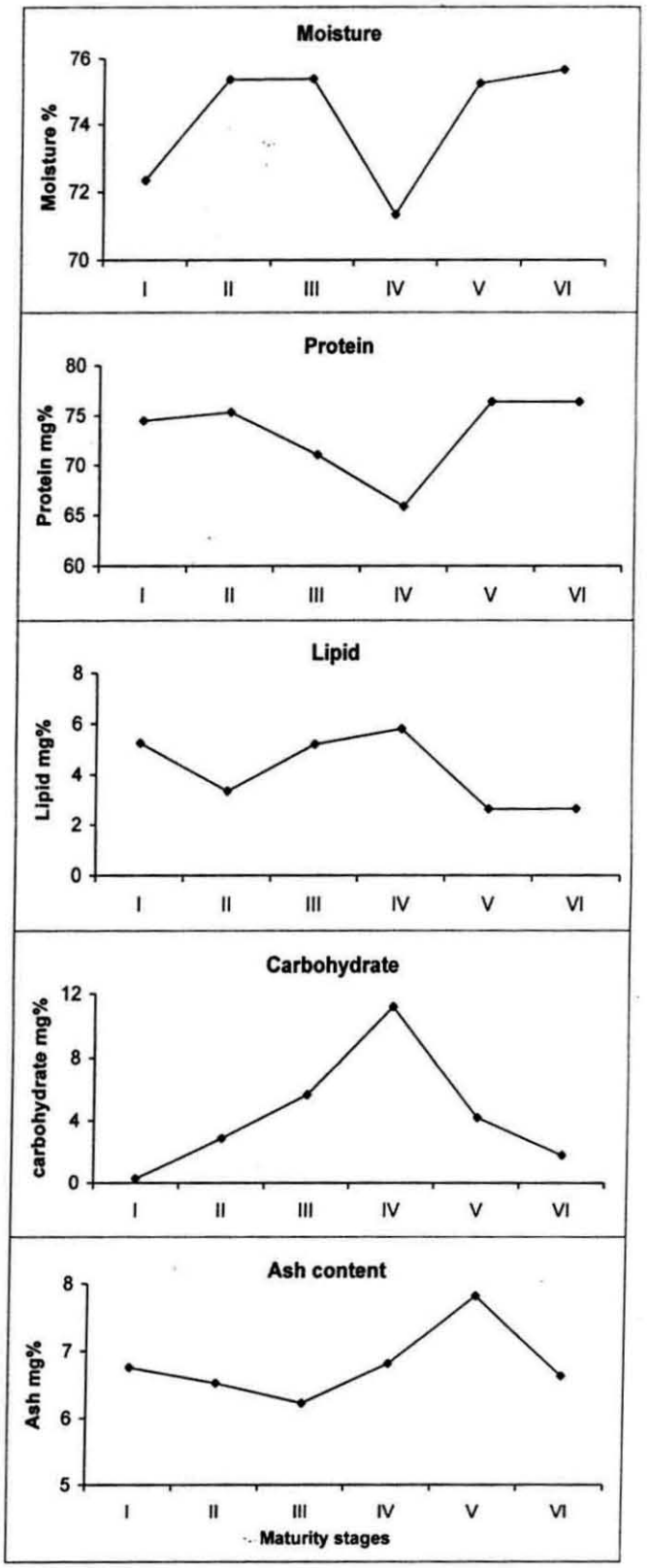


Fig. 28. Trends in the variation of biochemical components in the foot tissue of male *H. varia*

Table 24. Variations in the biochemical composition in the digestive gland of male *H. varia* (dry weight basis)

Maturity Stages	Moisture (%)	Total proteins (mg%)	Total lipids (mg%)	Total carbohydrates (mg%)	Ash (mg%)
Stage I	70.24 ± 1.50	53.80 ± 6.25	8.721 ± 0.970	9.881 ± 2.516	13.152 ± 0.353
Stage II	74.29 ± 2.72	55.17 ± 2.37	11.647 ± 1.283	10.647 ± 1.474	12.634 ± 0.331
Stage III	66.83 ± 3.27	52.18 ± 5.91	15.070 ± 3.143	11.268 ± 1.859	14.063 ± 1.276
Stage IV	64.00 ± 2.93	49.36 ± 2.94	9.130 ± 1.141	10.335 ± 1.379	13.778 ± 2.470
Stage V	67.09 ± 2.69	55.41 ± 8.53	10.667 ± 1.302	6.885 ± 1.949	16.792 ± 3.485
Stage VI	66.66 ± 3.67	50.41 ± 3.32	8.260 ± 2.066	13.527 ± 2.847	12.036 ± 1.818
F value $_{(5, 30)}$	39.416*	2.756**	15.196*	9.281*	69.480*

*: Significant at1% level (P<0.01), **: significant at 5% level (P<0.05)

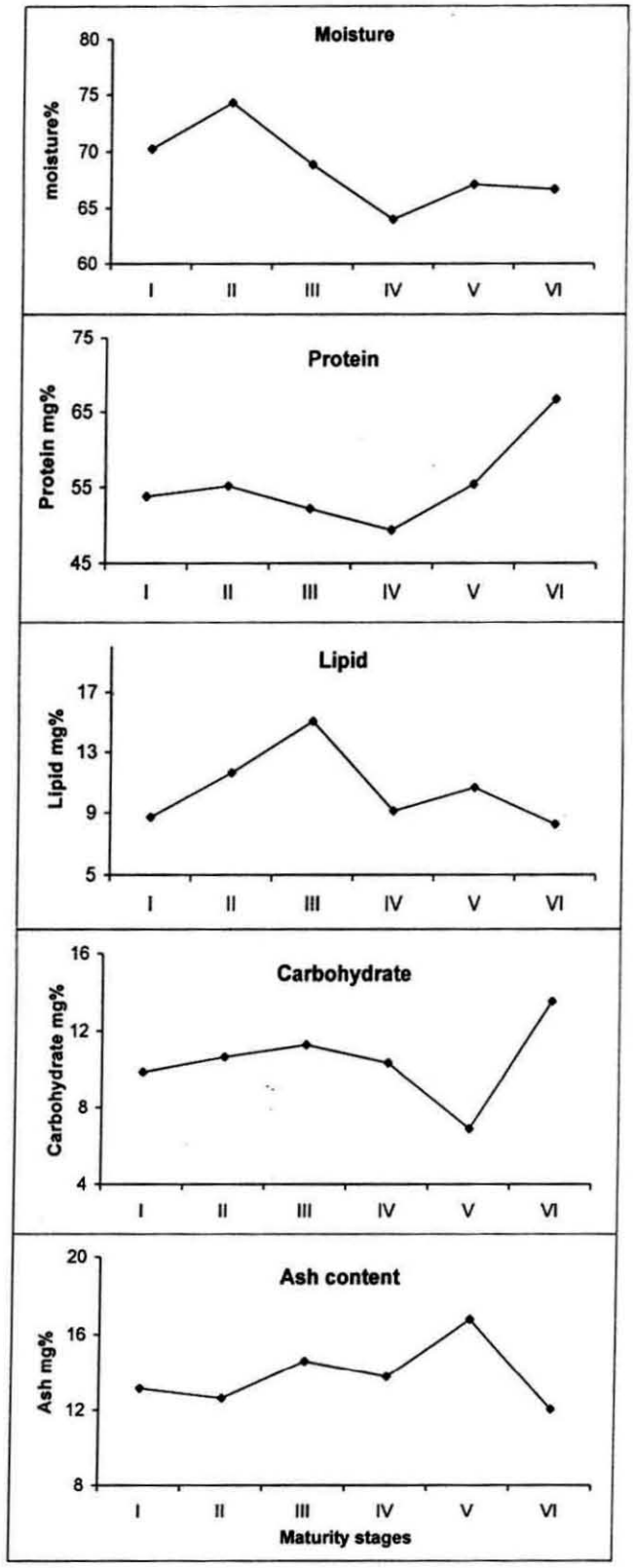


Fig. 29. Trends in the variation of biochemical components in the digestive gland tissue of male *H. varia*

Table 25. Variations in the biochemical composition in the gonad of male *H. varia* (dry weight basis)

Maturity Stages	Moisture (%)	Total proteins (mg%)	Total lipids (mg%)	Total carbohydrates (mg%)	Ash (mg%)
Stage I	75.54 ± 2.05	61.11 ± 6.01	9.154 ± 0.098	3.763 ± 2.104	7.635 ± 0.211
Stage II	77.23 ± 3.94	64.75 ± 2.90	5.527 ± 0.155	3.680 ± 0.300	9.023 ± 1.385
Stage III	74.03 ± 5.05	64.33 ± 3.40	9.837 ± 1.687	4.229 ± 0.652	9.635 ± 0.461
Stage IV	70.94 ± 4.74	54.92 ± 2.39	10.110 ± 1.583	1.736 ± 0.361	11.515 ± 1.192
Stage V	78.74 ± 6.37	60.31 ± 4.28	5.250 ± 1.062	2.237 ± 0.484	12.060 ± 2.210
Stage VI	74.23 ± 3.05	60.82 ± 3.46	6.148 ± 0.935	4.877 ± 0.695	6.980 ± 1.260
F value $_{(5, 30)}$	21.986*	4.637*	106.926*	9.133*	268.298*

*: Significant at1% level (P<0.01)

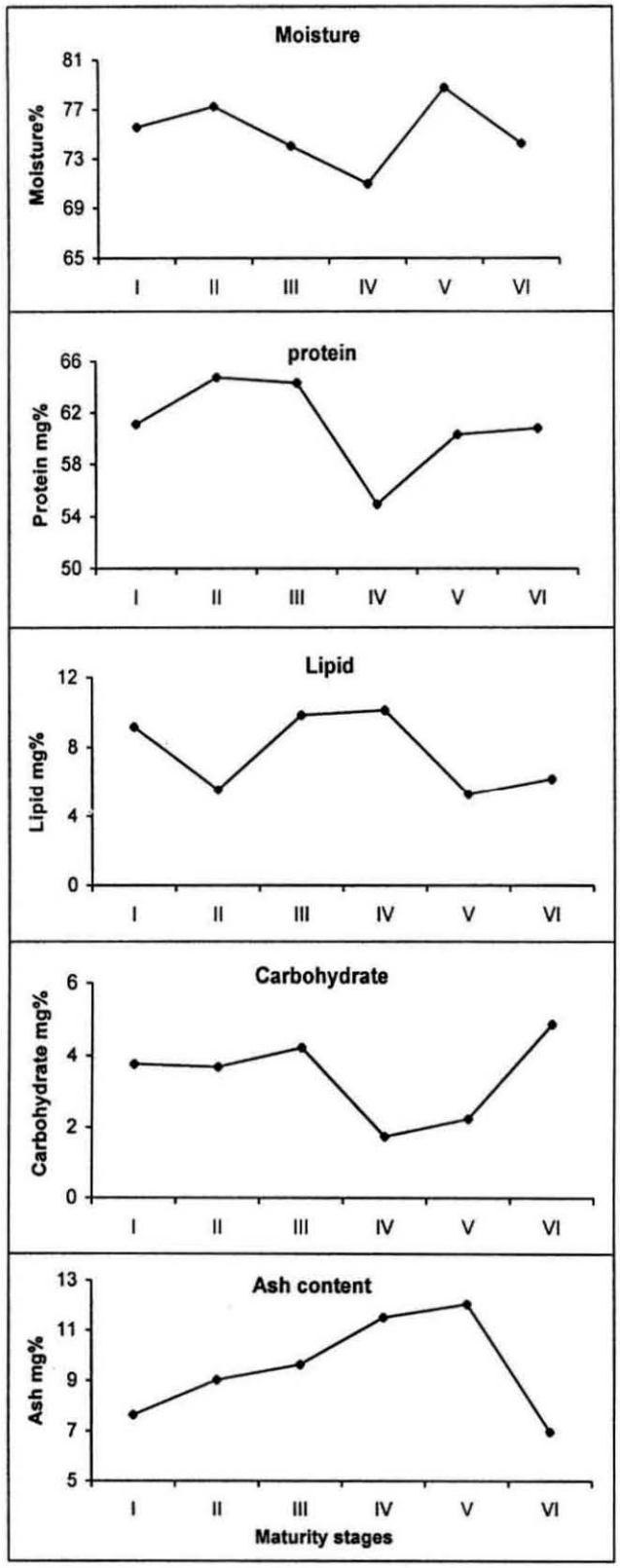


Fig. 30. Trends in the variation of biochemical components in the gonad tissue of male *H. varia*

DISCUSSION

Broadcast fertilisers devote much metabolic energy during their reproductive cycle for the production of gametes. The basic metabolic pattern in prosobranchs is very high lipid levels and low polysaccharide levels in the gonad and high polysaccharide and low lipid levels in muscular tissue, particularly the foot. The digestive gland section area of abalone usually remains fairly constant throughout the year as compared to the gonad section area, which fluctuates from month to month. However, the digestive gland will increase in size just before or immediately after spawning. Boolootian *et al.* (1962) found an inverse relationship between gonad size and digestive gland size, which indicated that material might be transported from digestive gland to the gonad.

Moisture forms the major constituent in animal body and plays decisive roles in most biochemical functions such as regulation of osmotic functions and as medium through which nutrients and other biochemical constituents are transported to various body parts. An animal may lose practically all of its fat and half its proteins and live, but loss of only 10% of its water causes death (Maynard and Loosti, 1962). Moisture content of any organism varies in accordance with its various physiological activities, and usually an equilibrium is established between water and other component systems.

High moisture level was observed in all the tissues analyzed throughout the course of maturation in *Haliotis varia*. Similarly such high values were observed in many of the molluscs analyzed (Joshi and Bal, 1965; Giese 1966; Webber, 1967; Ansell 1974; Nagabhushanam and Dheshmukh, 1974; Jeng *et al.*, 1979). More water was found in *Megathura* than in abalone; somewhat over 80% in muscle (Giese,

1969). Among the three tissue components analyzed, foot and testis had high amount of moisture in *H. varia*. In *Haliotis cracherodii* also the testis had high water content (73%) than foot tissue (70%) (Webber, 1967).

Moisture content in foot fluctuated irregularly throughout the course of gonad maturation and the fluctuation was insignificant both in male and female *H. varia*. Similar observations were reported in many molluscs. Moisture content in *Tivela* remained constant throughout the year for all of the body components (Giese *et al.*, 1967). The water content of the muscle showed little indication of a clear seasonal trend in *Chlamys septemradiata* (Ansell, 1974). The water content fluctuated between 79 and 82% through out the year. The male foot tissue had relatively low level of moisture than that in the female *H. varia*. In *Nucula nucula,* Ansell, (1974) reported the same trend with higher values in male foot than in the female.

In *H. varia*, the water content was relatively low in the ovary. High water content was observed in the spent ovary during the present investigation. Giese, (1966) observed that in *H. cracherodii*, moisture content is negatively correlated with lipid content. In *H. cracherodii*, the water level in the ovary falls considerably just before spawning when lipid level was high. Similar variations were noted in *Mopalia hindsii* (Giese, 1966b). The water level in ovary fluctuated insignificantly in *H. varia* as in *H. cracherodii* (Webber, 1967)

During the present study, the highest moisture content observed was in the testis. High moisture content in the testis was observed in *H. cracherodii* also. There was no regular trend of fluctuation in the water content as in Taiwanese clams where the moisture content was rather constant throughout the reproductive cycle (Jeng *et*

al., 1979). Giese (1966b) noted that the moisture content in *Katharina* spp. and *Cryptochiton* spp. was high (72-79%).

The lowest water content recorded for *H. varia* was in the digestive gland, which fluctuated irregularly throughout the reproductive cycle. In *H. varia*, as concluded by Giese (1966) in many other molluscs, a reciprocal relationship was found between water percentage and biochemical constituents. The lipid, carbohydrate and ash content were relatively higher in the digestive gland of H. *varia*, which might have caused the decrease of water content. In Taiwanese oysters, it is reported that lower moisture content was accompanied by higher protein and glycogen levels (Jeng *et al.*, 1979). Surprisingly low value (55.2%) was observed in the digestive gland of *Polinices lewisii* (Giese 1966). Water content in the digestive gland tissue remained relatively constant through out the reproductive cycle in *H. cracherodii* (Webber, 1967).

Protein was the major organic constituent, making up more than 50% of the dry weight of the various body components of *H. varia*. Similar results were obtained in the black abalone, *H. cracherodii* by Webber (1967). In the keyhole limpet, *Megathura*, it was even more that in the black abalone (Giese, 1966)

Of all the tissues analyzed, the highest value of protein was observed in the foot tissue, as noted in most bivalves and gastropods. In *Trochus somaticus*, the foot had a higher percentage of protein and less lipid and carbohydrate than the rest of the body (McLachlan and Lombard, 1980). Protein has been particularly prominent in the muscles of *Mopalia hindsii* (Giese and Araki, 1962). Data recorded on the protein levels for the red abalone *H. rufescens* were similar to those for the black abalone (Giese, 1966). Foot protein content in the present investigation showed significant

variation, but without any definite trend in relation to the stages of maturation both in male and female *H. varia*. So this could not be correlated with the process of vitellogenesis. Therefore, the irregular trend observed in the foot protein content might be due to its involvement in growth and metabolism rather than in reproduction. Identical conclusions were drawn by Giese *et al.* (1967) for *Tivela*, in which monthly variations in protein level had no relation to the reproductive season rather it was related to nutrient conditions and other variables in the environment. In *T. somaticus*, it is reported that high level of protein present (82%), must be utilized during stress (McLachlan and Lombard, 1980).

In *H. varia*, the protein content was relatively lower both in male and female gonads than that in the foot tissue. Protein levels were around 40 to 50% of the dry weight for both testes and ovaries of Chiton (Giese and Araki, 1962) and pismo clam (Giese *et al.*, 1967). In the testis of *H. varia*, the protein level increased to the maximum of 64.3% in the ripe stage and declined after spawning in the stage IV to 54.2%. But in the ovary, the value declined in the ripe stage. Similar observations were made in the male gonad of black scallop, *Chlamys varia*, in which the protein content increased more rapidly and usually contained greater amounts of protein than in females indicating the importance of nucleoproteins for male gametes (Shafee, 1981). In the bivalve molluscs also the protein levels of the gonads are quite variable and are highest when they are gravid which presumably reflects the high protein content of the maturing gametes (Giese, 1966). In *Trochus somaticus*, protein seems to be associated with gonad development and may be used to synthesize carbohydrate or lipid (McLachlan and Lombard, 1980). In *Katharina*, in the ovary and testis, the protein level shows marked variations; it reaches a peak during the height of

the reproductive season in spring and then declines to the minimum after spawning (Giese and Hart, 1967). The low level of protein in the ripe ovary during the present study may be due to the high level of lipid content during the ripe phase of the ovary. McLachlan and Lombard (1980) reported that in *T. somaticus*, lipid was negatively correlated with protein. In most bivalves the protein level remains at a relatively high levels except during the breeding season (Durve and Bal, 1961; Deshmukh, 1972). The level of protein build up during gametogenesis in *Villorita cyprinoides* is utilized during the breeding season by the animal (Ansari *et al.*, 1981).

In the digestive gland of *H. varia*, the protein content was lowest in all the tissues analyzed. The same observation was found in the keyhole limpet *Megathura*, in which the least protein is present in the digestive gland (Giese, 1966). High level of lipid and carbohydrate present in the digestive gland throughout the course of gonad maturation may be the cause of this low protein value. The fluctuation of protein value in the digestive gland of male had no definite trend in relation to the stages of maturation. Similarly, in the digestive gland of *H. cracherodii*, protein levels did not show any consistent change relative to the gonad cycle. In *H. varia*, the protein content in the female decreased in the ripe stage, indicating the transport of protein yolk from the digestive gland to the ovary. In gastropods ferritin is an important component of yolk (Fioroni and Schmekel, 1976). In most species, the only extra gonadal protein accumulated in yolk granules is ferritin. This protein is reported to be derived from the digestive gland (Deschiens *et al.*, 1957; Johnson *et al.*, 1962; Heneine *et al.*, 1969).

Lipid value of the ripe ovary of *H. varia* was similar to those of the ovary of *H. cracherodii* and Chiton ovary. Lowest lipid values were observed in the foot tissue

of both male and female *H. varia*. The values remained more or less static throughout the reproductive cycle with a small peak in the ripe stage. Such low lipid levels were observed in *Meretrix meretrix* by Nagabhushanam and Deshmukh (1974).

The abalone gonad lipid levels follow the pattern described for other molluscan broadcast fertilizers in that ovary lipid levels are about twice the testis lipid levels. In all the gastropods mentioned, lipid is apparently the nutritive storage product of the eggs. In the present study, the ovary lipid levels were high and steadily increased from stage I to the stage III and then declined to the lowest values after spawning. In the females, there was a greater increase in lipid content with the result that ripe female gonad contained approximately three times as much lipid as male, indicating the importance of lipid as an energy source for the planktonic eggs and larvae of marine gastropods. Albrecht (1923) reported high lipid values for the abalone. In the wood boring mollusc, *Martesia fragilis*, lipid is reported to constitute as much as 58.5% of the dry weight in young animals (Giese 1966). Similar observations were made in the red abalone also. The presence of lipid yolk granules in the cytoplasm of ripe oocyte of *H. varia* has already been revealed in the present study using electron microscopy. High ovary lipid levels have been reported in several bivalves and gastropods (Shafee, 1981; Ansell *et al.*, 1964). Ansell *et al.* (1980) reported that the greatest increase in the total lipid content was associated with the period of vitellogenesis in *Donax trunculus*. Similar observation was made by Victor (1984) in *D. cuneatus*. Conversely, the lipid content of the testis was considerably low in *H. varia*. It was same in most of the molluscs reported (Shafee, 1981; Victor, 1984; Giese, 1967). Fluctuation in the lipid content of testis was not correlated with the process of testis maturation.

H. Varia is similar to *H. cracherodii* (Webber, 1967) and Chiton (Giese and Araki, 1962) with low immature gonad lipid levels compared with the mature gonads. In oysters and clams immature ovary and testis have low lipid and high carbohydrate values (Galtsoff, 1964; Giese *et al.*, 1967).

The digestive gland of *H. varia* contained relatively high amount of lipid throughout the period of gonadal maturation. Similarly, lipid levels reported are surprisingly high in the squid digestive gland (Giese, 1966). Lipid is prominent in the ovary and the digestive gland of *Cryptochiton.* It was much prominent in the digestive gland of *Meretrix meretrix* (Nagabhushanam and Deshmukh, 1974). Giese *et al.* (1967) observed that digestive gland of *Tivela* contained more than 10% of wet weight as lipid and it was relatively stable throughout the reproductive cycle.

In *H. varia*, the carbohydrate levels in the gonad and foot were low throughout the course of gametogenesis. In *H. cracherodii*, the carbohydrate in the foot tissue was found to be apparently glycogen (Webber, 1967). But only less than one half of the polysaccharide levels of gonad and digestive gland tissue was glycogen. In the present study, carbohydrate levels were lower in foot tissue and in the gonad and higher in the digestive gland. Similar observations were reported in the black scallop, *Chlamys varia* (Shafee, 1981), where the gonad contained relatively little glycogen. In the female foot tissue, carbohydrate value increased in the stage III and IV, indicating the glycogen content in the foot tissue and began to decrease after spawning and was low in the spent stage. This is in agreement with the observations made in the Taiwanese oysters and clams (Jeng *et al.*, 1979).

In *H. cracherodii*, Webber (1967) reported that during post spawning gametogenesis foot size and glycogen levels increased. They explained this

difference in pattern to the increased feeding activity of abalone during the post-spawning period than at any other time during the reproductive cycle. Such increase in carbohydrate value was not observed in *H. varia* during the present study.

The total carbohydrate values of abalone gonads fit into the range of other molluscs, but the variability between the species are larger. The gonads of the limpet, *Patella* has very low glycogen (Barry and Munday, 1959). Mature Chiton gonads have less than 1% carbohydrate (Giese and Araki, 1962). In the present study, the carbohydrate content in the gonad were low throughout the period of gametogenesis, which is similar to the above examples. Similarly, low carbohydrate value is reported in the gonad of *H. cracherodii* (Webber, 1967). Oyster and clam gonads, on the other hand, appear to be having well-developed nutritive storage areas that are involved with providing gametogenesis (Giese, 1966).

The gonad carbohydrate content was low when the lipid content was high. Tanaka and Hatano (1952) and Masumoto *et al.* (1934) have shown that as lamellibranch gonad lipid levels rise, glycogen levels decrease. In *Crassostrea gigas* (Hatanaka, 1940) and *Pinctada martensi* (Ashikaga, 1948), it was reported that carbohydrate was converted to lipid during gametogenesis. The same pattern was observed in the present study in that the gonad carbohydrate level decreased when lipid level increased. A similar synchrony of glycogen breakdown and vitellogenesis has been recorded for *Mytilus edulis* by Lubet (1959) and Gabbott and Bayne (1973). Low carbohydrate values for the spent gonads observed here are comparable with the results in the black scallop, *Chlamys varia* (Shafee, 1981). Lambert and Dehnel (1973) found that glycogen was used in the normal synthesis of gonad material in *Thais lamellosa*.

The digestive gland carbohydrate values were high during the course of gonad maturation, both in male and female *H. varia*. This is in contrast to the observation in *H. cracherodii* (Webber, 1967), where digestive gland carbohydrate levels were low throughout the reproductive cycle.

In the foot tissue of *H. varia*, the ash content remained more or less static throughout the period of gametogenesis. The fluctuation was insignificant. Similarly, no consistent variation in ash values over the reproductive cycle was observed in *H. cracherodii* (Webber, 1967). As in the present results, the ash levels in most of the body components of *Loligo opalescens* were relatively low (Giese, 1966). In the black scallop, *Chlamys varia*, the ash content of the somatic tissues showed irregular fluctuations as in *H. varia*, in relation to the reproductive cycle.

The gonad ash level was little higher than that in the foot tissue. Testis contained more ash content than in the ovary *of H. varia*. Similar observations were reported in many other studies. In *H. cracherodii*, testis had higher ash values at around 7% of the body weight. The ash level in the indeterminate gonad is comparable to the ash levels in the ovary in *H. varia*. Similar trend is reported in *Tivela* by Giese (1966). In the present study, in the testis, high value of ash was observed in the spent stage of *H. varia*. Similarly, the percentage of ash content was maximal in spent gonads, and showed low values in ripe gonads of *Chlamys varia* (Shafee, 1981).

Among all the tissues, digestive gland had the highest ash content in *H. varia*. The highest ash value was in the spent male digestive gland. This may be due to the accumulation of minerals in the digestive gland tissue. High ash values are reported in the digestive gland of *H. cracherodii*, *Megathura, Tivela, Modiolus* and *Cryptochiton*

(Giese, 1969). In gastropods, ferritin, one of the yolk precursor materials containing iron, is produced in the digestive gland and is incorporated into the ovary prior to vitellogenesis (Fioroni and Schmekel, 1976; Bottke, 1977). In *Tivela*, it is reported that ash levels remained relatively low, and rose with increase in GSI. Conversely, in the present study ash value increased with decrease in GSI, i.e., in the spent stage. Only the digestive gland ash content fluctuated significantly throughout the period of gametogenesis.

Chapter IV

SEED PRODUCTION

1. Introduction
2. Materials and Methods
3. Results
4. Discussion

INTRODUCTION

Abalone Sea farming targeted for its meat has spanned nearly half a decade. In recent years due to the overexploitation of wild abalones, the world production of abalones through capture is gradually declining. This led to the development of culture techniques for abalones. The commercial demand for abalone and the existence of a farming technology have led to the development of abalone farming as a commercial venture. Seed production of this mollusc has been developed on a large scale for maintaining and augmenting the resource.

During the 1970s there was surge in valuable contributions on various aspects of abalone cultivation (Leighton, 1972, 1974, 1977; Shibui, 1972; Kikuchi and Uki, 1974a,b,c; Kan-no, 1975; Morse *et al.*,1977, 1979b; Seki and Kan-no, 1977, 1981a). As an outgrowth of these investigations, interest in cultivating abalone, an economically valuable shellfish, has spread from Japan to various parts of the world. Research and developmental projects or production hatcheries, are in operation in Australia, the British Isles, Canada, Chile, France, Mexico and the United States. Japan is at present the acknowledged leader in developing techniques for the mass production of juvenile abalones. No successful rearing of *Haliotis varia* has been reported to date, whereas most of the commercially important species in other countries have been cultured and their larval and post larval development documented (Ino, 1952, Leighton, 1974).

Literature on the inducement of spawning, fertilisation, larval rearing and inducement of settlement and metamorphosis in Haliotids is vast. Artificial inducement of spawning is usually the first step in developing seed production techniques of a new

species. Several endogenous and exogenous factors such as sudden change in water temperature, aerial exposure during low tide, photoperiod, lunar cycle, release of gametes from other individuals in the population, or some combination of these or others have been cited as the cause for triggering spawning. Spawning inducement by desiccation method has been successfully achieved in *H. rufescens* by Carlisle (1945). Ino (1952) was the first person to use thermal shock to induce spawning. Koike (1978) used the desiccation and thermal shock experienced by wild caught *H. tuberculata* brood stock during transport to the laboratory as the method of induced spawning.

The use of UV light to induce spawning was an accidental discovery by Shogo Kikuchi and Nagahisa Uki at the Tohoku Regional Fisheries Research Laboratory. UV irradiation is believed to cause the energetic decomposition of the water molecule to produce a hydroperoxy free radical, HOO·, or peroxy diradical ·OO· (Morse *et al.*, 1977, Uki and Kikuchi, 1984). Induced spawning with UV irradiation has been successfully achieved in *H. discus hannai* (Kim and Cho, 1976; Uki and Kikuchi, 1984), *H. rufescens* (Ebert and Houk, 1984), *H. diversicolor supertexta* (Liu *et al.*, 1987) and *H. diversicolor* (Bryan and Qian, 1998).

The hydroperoxy free radical HOO- or peroxy diradical can be produced chemically by the addition of peroxide to water. Hydrogen peroxide is the cheapest and the most commonly used peroxide for the inducement of spawning (Morse *et al.*, 1977, 1978). Spawning inducement with hydrogen peroxide has been successfully accomplished in *H. rufescens* (Morse, 1979, 1984) and in *H. coccinea canariensis* (Pena, 1975).

Although the hormonal control of reproduction in abalone and other prosobranch gastropods is unknown, it is suspected that a prostaglandin like chemical may be involved in the inducement of spawning. Morse *et al.* (1977) was able to induce spawning by adding the invertebrate hormones prostaglandin E or F to the water. Yahata (1973) found that the pedal-pleural and visceral ganglia were involved in inducing spawning in *H. discus hannai.*

Fertilisation of the gametes is a critical step in the hatchery process. If all the eggs are fertilised in a narrow time period, the larval development will be almost synchronous (Kikuchi and Uki, 1974c). The most common cause of poor fertilisation is improper sperm concentration.

Larval development in *Haliotis* spp. occurs at a faster rate with high water temperatures. Seki and Kan-no (1977) were the first researchers to calculate the critical minimum water temperature for larval development of an abalone species. Leighton (1974) found that larvae could be cultured from 10 to 25 °C, with the best development at higher water temperatures. Forty-one distinct larval stages with recognizable external features reported in *H. discus hannai.* Larval development of *H. discus* has been described by Ino (1980). The larval developments in *H. sorensini* (Leighton, 1972), *H. tuberculata* (Koike, 1978) and *H. kamtschatkana* (Beaudry, 1983) have been studied in detail.

During the rearing period, many factors can cause high mortality, such as: sperm and egg preservation time (Kikuchi and Uki, 1974a), sperm density (Kikuchi and Uki, 1974c; Vacquier *et al.*, 1980); hydrographic conditions of rearing medium like temperature (Leighton, 1972; Ebert, 1974), salinity (Yang, 1979) and dissolved oxygen

and stocking density (Sagara and Arochi, 1971). The general water quality is also reported to play significant role (Tanaka, 1969; Morse, 1979).

The larvae of many benthic marine invertebrates depend strictly upon recognition of external chemical signals from the environment for activation of their genetically programmed settlement and metamorphosis. The natural inducer of this process in *H. rufescens* is a GABA-mimetic peptide that larvae normally encounter at the surface of the crustose red algae of the genera *Lithothamnium, Lithophyllum* and *Hildenbranchia* (Morse and Morse, 1984). Coralline algal crusts induce metamorphosis of the larvae of numerous invertebrate taxa and in particular, those of many molluscan species (Barnes and Goner, 1973; Rumrill and Cameron, 1983), including abalone (Morse *et al.*, 1979a; Morse 1984; Morse and Morse, 1984; Shepherd and Turner, 1985; Prince *et al.*, 1987). *Haliotis* larvae also can be induced to settle out of the water column, attach to the substrate and metamorphose into their juvenile form by γ-amino butyric acid (GABA) and by GABA analogs (Morse *et al.*, 1979a,b, 1980b,c).

Biotic films derived from ambient seawater or the mucous trails of grazing adult abalone are routinely used to induce metamorphosis (Seki and Kan-no, 1981a,b; McCormick and Hahn, 1983; Toole, 1988; Hahn 1989b). Regulation of receptor-mediated settlement and metamorphosis in larvae of *H. rufescens* has been reported by Trapido-Rosenthal and Morse (1986). Inducement of larval attachment and metamorphosis in *H. diversicolor* has been achieved by Bryan and Qian (1998).

As the larvae of the Haliotidae are lecithotrophic (Leighton 1974), no food is given until the settlement stage is reached. Benthic diatoms are a principal food source for post larval abalone (Kawamura, 1996). Norman-Boudreau *et al.* (1986)

developed a technique to determine the diatom species eaten by newly settled juveniles. *Navicula* spp. and *Nitzschia* spp. are the usual diatom species given to juvenile abalones as feed (Ino, 1980; Hooker and Morse, 1985). Moreover, larvae of haliotids are also found to attach to the substratum and metamorphose in the presence of diatoms and conspecific mucus (Seki and Kan-no, 1981b; Slattery, 1992). Maintaining a suitable diatom film is a critical factor in the success of abalone hatcheries worldwide.

Substantial work has been done in the last two decades on the spawning, larval rearing and the seed production of abalones worldwide. Seed production and rearing of the abalone in controlled conditions has been demonstrated by Lee *et al.* (1978). Chen and Yang (1979) investigated on the artificial propagation of *H. diversicolor supertexta* in China. Preliminary study on the artificial breeding of *H. discus hannai* was carried out by Du and Guo (1981) and Pyen *at al.* (1981). Spawning and rearing of Paua, *H. iris* has been demonstrated by Tong (1982). Beaudry (1983) investigated the survival and growth of the *H. kamtschatkana* larvae at different temperatures. The effects of salinity, temperature and food on the development of the fertilized eggs of *H. discus* hannai were studied by Nie *et al* (1984) and Nie and Chen (1984). Pena (1984) studied the egg and larval development of *H. discus.* Larval rearing and seed production of *H. rufescens* in controlled conditions has been demonstrated by Ebert and Houk (1984). Trapido-Rosenthal and Morse (1985) has investigated the receptor-mediated settlement and metamorphosis of *H. rufescence* larvae in the laboratory. Norman-Boudreau (1986) worked on the feeding of the newly metamorphosed larvae of abalone. Enhancement of natural population of the abalone *H. iris* using cultured larvae was achieved by Tong *et al* (1987). Genade *et al* (1988) achieved controlled

spawning and development of the South African abalone *H. midae*. The juvenile of *H. leavigata* has been successfully produced in Australia by McShane (1988). Jaeckle and Manahan (1989a) studied the influence of water quality on the larvae of red abalone. The same authors, in another study, (Jaeckle and Manahan, 1989b) shown that the uptake of dissolved amino acids by the lecithotrophic larvae of *H. rufescens* from sea water. The effect of sperm density on the fertilization rate of abalone was studied by Gao *et al* (1990a). Gao *et al* (1990b) investigated the influence of temperature on the feeding and growth of the juvenile abalones. Morse and Morse (1991) have unveiled the molecular signals involved in reproduction and development and growth of molluscan larvae and their role in aquaculture. lang *et al* (1992) have developed the techniques for the culture of paua, *H. iris* to the early juvenile stage. Optimum sperm density for successful fertilization has been studied by Mill and McCormick (1992). Seed production and culture of the tropical abalone *H. asinina* has been studied by Tenin and Masanori (1993). Embryonic and larval development of *H. ovina* were given by Jarayabhand *et al* (1995).

Chemical cues involved in the settlement of abalone larvae were studied by Morse (1990). Effect of larval stage on the settlement of the abalone *H. iris* has been studied by Moss and Tong (1992). Degnan and Morse (1995) demonstrated the morphogenic and developmental gene regulation in *H. rufescens* larvae. Yang and Wu (1995) has successfully induced the larval settlement and metamorphosis in *H. discus hannai*. The role of benthic diatoms in the early stages of *H. discus hannai* has been studied by Kawamura (1996). Jarayaband and Paphavasit (1996) reviewed the culture of the tropical abalone *H. asinina* with special reference to Thailand. Survival and growth of post larval *H. discus hannai* fed, conspecific trail mucus and

diatoms have been studied by Takami *et al.* (1997). Factors affecting the food value of diatom strains for abalone post larvae have been studied by Kawamura *et al.* (1998). Buchal *et al.* (1998) have used Dulse, pulmaria mollis as a settlement substrate and food for the red abalone *H. rufescens*.

Owing to its importance in the world aquaculture scenario, it is important to initiate research on abalone culture in India with the native species. The restricted and moderate distribution of abalones necessitates production through aquaculture. The first step in abalone culture is to standardize the techniques for seed production including spawning, fertilization and hatching, larval rearing, inducement of settlement and metamorphosis and the production of juveniles with respiratory pores. The purpose of this investigation is to demonstrate that spawning, larval development and further growth of *Haliotis varia* can be achieved under controlled and semi-controlled conditions.

MATERIALS AND METHODS

1. BROOD STOCK MANAGEMENT

1.1 COLLECTION AND TRANSPORTATION

Matured male and female abalones of more than 25 mm in shell length were collected with the aid of a wooden chisel from the intertidal rocks of the Tuticorin harbor basin, mostly during full moon and new moon days. Care was taken not to damage the foot while dislodging them from the rocks. The live spawners were then transported from Tuticorin to the Mandapam Regional Centre laboratory of CMFRI by road during late hours as mentioned in the chapter II.

1.2. MAITENANCE

The transported abalones were stocked in 1.5 ton FRP tanks filled with filtered sea water. The salinity variation between the site of collection and the tank was kept at less than 5 ppt. Finely chopped thin pieces of freshly collected seaweed *Ulva lactuca* and *Polysiphonia* spp. were given as feed. Half of the water was replaced every day and the wastes accumulated in the bottom of the tank were siphoned out.

2. SPAWNING INDUCEMENT AND FERTILISATION

Spawning inducement was accomplished by the desiccation method (Carlisle, 1945). For this, the ripe males and females were exposed to air for 2 hours before they were transferred to the spawning container. The animals were kept in a plastic basin containing 30 liters of clean filtered sea water of 30 ± 2 ppt. Salinity. Three sets of containers were used for spawning, larval rearing and sttlement experiments. Two pairs of abalones in the ratio 1:1 male to female, were placed in one container. When successful spawning was not observed, sperms of abalones were introduced in to the

spawning container by teasing the ripe male gonad. The presence of sperms in the container was found to induce the ripe animals to spawn. In general, spawning occurred at late night hours or early morning hours when the temperature was around 25 °C.

After the gametes were completely extruded from the gonad, the container was left undisturbed for one hour to facilitate fertilization. In the fertilized eggs the perivitelline space between the oolemma and the vitelline layer increased in size and the eggs settled to the bottom of the container.

The fertilized eggs were collected by siphoning out the bottom water through a 50 μm sieve, which was followed by repeated washing with clean filtered sea water to remove the excess sperms. After estimating the fertilization percentage, the eggs were transferred to another tank with filtered sea water for hatching.

3. HATCHING AND LARVAL REARING

The fertilized ova developed to the early veliger stage in the hatching container, which were positively phototactic with a tendency to congregate at the water surface. The swimming larvae were siphoned out to a container with 20 liters of filtered sea water. The bottom water of the hatching container with the discharged egg membranes, unhatched eggs, detritus and abnormal larvae were discarded. The density of the larvae in the rearing container was estimated by aliquot sampling. For this, a uniform larval distribution was obtained by stirring the water column with a glass pipette. Aliquot samples were taken with a 1 ml pipette. Three to five aliquots were taken depending upon the variation in larval counts between samples. If the variation exceeded 50%, then five aliquots were taken.

As the larvae of Haliotidae are lecithotrophic (Leighton, 1974), no food was added to the larval rearing containers until the settlement stage was reached. Also, water flow and aeration were stopped during larval development. The water was changed once a day with clean filtered sea water after siphoning the larvae on to a 50 µm net. Larval developmental stages were recorded twice daily by microscopic examination of the larvae. The different stages of embryonic development and larvae were photographed using a binocular compound microscope (Olympus, Japan) with a camera unit. Photographs were taken using Konica 200 ASA color negative films.

4. SETTLEMENT, METAMORPHOSIS AND DEVELOPMENT TO EARLY JUVENILE STAGE

On day 4, the floating veliger began settle on substrates. The larvae were transferred to the settling containers. The settling containers were of 20-liter capacity plastic or glass tanks with a thin and uniform layer of benthic diatoms. The settling containers were placed in a green house that incorporates translucent plastic siding in order to maximize solar illumination. The larvae were introduced to the settling containers on the 4th day of hatching. No aeration was provided in the containers. Water exchange was done twice daily with clean filtered sea water after filtering through 100 µm mesh net. The sea water in some of the larval rearing and settling containers was treated with antibiotics to reduce mortality of the larvae.

After the successful settlement of the creeping larvae, the settled spats were thinned and introduced to another container of 20-liter capacity for metamorphosis. The spats were removed from the walls of the container carefully using a thin small paintbrush. No anesthetics were used to remove the spats from the walls of the settling container. The containers were covered and uncovered periodically to regulate

diatom growth. The success of the metamorphosis was recorded by examining the settled spats at regular intervals under a compound microscope.

5. DIATOM CULTURE

The culture of the diatom mat, which was necessary for the settlement and metamorphosis of the gliding larvae was commenced 4 days before the larval seeding. Benthic diatoms like *Nitzschia* spp., *Navicula* spp., scraped from the inner walls of the containers used to store sea water were used as inoculam. 20 liters of sea water in plastic containers enriched with Walne's algal culture medium (Walne, 1974), was seeded with scraped out diatom. The containers were then kept in diffused sunlight without aeration. After 3 to 4 days, a uniform thin layer of diatoms was formed along the walls of the container. The water of the container alone was changed on alternative days to keep the diatom mat healthy.

6. JUVENILE CULTURE

After the metamorphosis of the abalone spat, the juveniles with 1 to 2 respiratory pores were transferred to the culture tanks. The juveniles were cultured in 20-liters capacity plastic containers filled with clean filtered sea water. The mat of diatoms was retained on the walls of the juvenile culture tank. Supplementary food was provided as fresh thin pieces of *Ulva lactuca* in some of the tanks. The other tanks were provided with coralline red algae attached to coral stones (Plate XVII3) and green filamentous algae, as food. The water was changed once in 3 days with clean filtered sea water. The growth of the juveniles was assessed weekly by measuring the shell length and width with vernier calipers. The juveniles were cultured in the above tanks till they reached about 15 mm in shell length.

RESULTS

1. BROOD STOCK MANAGEMENT

During the breeding season, *Haliotis varia* attains full sexual maturity on full moon or new moon days. The animals collected on the previous day of full moon or new moon day had fully mature gonads. During collection using chisel, damage was inflicted to the foot, which caused mortality during transportation. Around 40 ripe males or females were usually collected for spawning experiment. The transportation time from the collection site to the laboratory was 6 to 8 hours. The transportation was carried out during evening or early morning hours when the temperature was low. More than 80% survival was achieved by this method. The mortality of the animals noticed was mostly due to the damage caused to the foot on collection.

The transported abalones were placed in 1.5-ton capacity FRP tanks. If the salinity variation between the site of collection and the sea water in the tank was more than 5 ppt, high mortality was observed. When water exchange was done frequently, gravid animals spawned in the tank itself. So the sea water in the brood stock maintenance tank was kept undisturbed for maintaining the condition of the gonad.

2. SPAWNING INDUCEMENT AND FERTILIZATION

Natural spawning commenced in the Tuticorin stock of *H. varia* from November and peak spawning was observed during January and February. The desiccation method of spawning was found to be successful in the laboratory when the animals were fully mature. The abalones that were exposed to air for 2 hours yielded the most spawnings. The fully ripe male gonad was creamy white and in the females it was dark blue in colour. Spawning usually occurred at late night or early morning hours

when the temperature was around 25 ºC. Male spawned first followed by the females. The presence of sperms in the spawning container triggered the other males to spawn, which in turn triggered the females to spawn. The partially spawned animals were again used for another spawning trial. When sperms were introduced to the spawning container by teasing ripe male gonad, the male abalones spawned within one hour. Animals kept during night hours for spawning obtained good result. Mostly the spawning was observed in the early morning. Ejaculation of ova and sperm occurred mainly through four respiratory pores. In contrast to the ova, which settled to the bottom within a few minutes, the sperm stayed in suspension.

Spawning experiment was tried by adding hydrogen peroxide to the spawning container. Because the animals used for the above experiment were not fully ripe, no good result was obtained. However, only males responded to this treatment.

The eggs were fertilized within one hour of spawning. The fertilized eggs, 180-200 µm in diameter were spherical in shape (Plate XVIII1). They immediately absorbed water and sank to the bottom of the container. After fertilization, the perivitelline space between the vitelline layer and the egg plasma membrane increased in size. The fertilized eggs were collected by siphoning out the water at the bottom of the spawning container and filtered through a 50 µm sieve. Excess sperms were found to be detrimental to the success of hatching. Repeated washing with clean filtered sea water during filtration removed the excess sperms. The average fertilization percentage obtained in the three sets of experiment was 40-60%. The unfertilized eggs were spherical and enclosed within a 300-400 µm thick gelatinous coating and floated on the surface.

3. HATCHING

Cleavage began after the extrusion of polar bodies and the egg developed further to reach the blastula stage. The development at 27 °C proceeded to the 2-cell stage in 2 hours (Plate XVIII3). The next few divisions occurred at hourly intervals. The embryo passed the two, four, and eight-cell stage and finally the gastrula (Plate XVIII4). At about 10 hours of post-fertilisation, the trochophore larva completed its development inside the egg membrane and began to rotate (Plate XVIII5). In about 12 hours, the trochophore larva ruptured the egg membrane (Plate XVIII6) and swam upward in the water column. The average hatching percentage in the experiments was around 70%.

4. LARVAL REARING

The trochophore larvae were positively phototactic and had a tendency to congregate at the water surface. These swimming larvae were siphoned out to the larval rearing container, with clean filtered seawater. The larval stages of *H. varia*, till settlement are described below.

5. LARVAL STAGES OF *H.VARIA*

5.1. TROCHOPHORE LARVA

Trochophore is the first larval stage of *H. varia*. During the embryonic development, cilia grew along the top of the embryo forming the prototrochal girdle and apical tuft, and began beating. The cilia cause the embryo to rotate intermittently inside the egg membrane (Plate XVIII5). The stomodeum is formed and cilia along the prototrochal girdle are completely formed. At this stage the embryo is classified as trochophore larva. The larva begins moving more frequently inside the egg membrane, the egg membrane becomes thinner and finally bursts (Plate XVIII6). The

apical cilia aid the larva in bursting the egg membrane. The hatched trochophore immediately swims to the water surface. The trochophore larva is positively phototactic and has a tendency to congregate at the surface of the hatching container. They measure around 180-200 μm in length. The free-swimming trochophore larval period of *H. varia* extends only for 10 to12 hours at 27 °C.

5.2. EARLY VELIGER LARVA

The veliger larval stage starts at about 24 hours of post-fertilisation in *H. varia*. The trochophore larva continues to develop until it becomes a veliger larva. The veliger stage is reached when the apical region of the larva becomes flat and the velum is completely developed with long cilia present (Plate XIX1). The larval shell grows from dorsal to ventral, until it covers the body just below the velum. The behaviour of veliger is same as that of the trochophore larva. Veliger larvae also show positive phototaxism. The early veligers measure 210 ± 12 μm in length.

5.3. LATE VELIGER LARVA

In the late veliger larvae, the foot mass protrudes to the top of the shell at the completion of the larval shell (Plate XIX3). During torsion, the velum and cephalo-pedal mass rotate between the region of the body covered by the larval shell and the waist. The region destined to become the mouth and the foot continues to rotate, until the cephalo-pedal mass is rotated 180° from its original position. The late veliger stage of *H. varia* extends to the 3rd day of post-fertilisation, until it reaches the gliding stage.

5.4. GLIDING LARVA

On day 4 of post-fertilisation the floating veligers began to settle on substrates. At this stage, they had the cephalic tentacles with four branches and well-developed eyespots. The foot sufficiently developed and the veliger could pull itself upright and

also propel itself by ciliary action. This stage, termed as the gliding stage (Plate XIX4) most suited for transferring to the settling containers. The larva actively crawls with its foot, but does not stop swimming behaviour unless suitable settlement substratum is present.

5.5. CREEPING LARVA

Although the gliding larva shows crawling behaviour after the formation of first epipodal tentacle, it is not until the fourth tubule is formed on each of the cephalic tentacles that the gliding larva shows the crawling, exploratory movements characteristic of settling larva. Shortly after, the cilia disappear and the larva strictly depends on the substratum and crawls actively. This is the creeping stage and the larval development is completed with the formation of fourth tubule in the cephalic tentacle. This stage onwards, the larvae required suitable food in sufficient quantities. A mat of benthic diatoms comprising mainly of *Navicula* spp. and *Nitzschia* spp. was found to be the ideal food. For *Haliotis varia*, the larval rearing period ranged from 4 to 5 days when the water temperature was 27 °C. The creeping larva measured 260 ± 12 μm in length.

6. INDUCEMENT OF SETTLEMENT AND METAM0RPHOSIS

On the 5th day of post-fertilisation, most of the larvae ceased swimming and crawled over the substratum of the diatom mat along the walls of the settling container. Majority of the larvae settled on the vertical side of the container. No settlement was observed on the bottom of the container. After this, the larvae seldom detached themselves from the diatom mat. Complete settlement was observed on 7th day of post-fertilisation at 27 ± 2 °C (Plate XIX5).

Peristomeal growth, the first step in the metamorphosis, started on the 6th day leading to the transformation of the round tubular larval shell to one, which resemble the flat abalone shell (Plate XIX6). The animal at this stage actively feeds on the diatoms and clears the diatom mat areas by moving with their muscular foot. It was observed that mortality occur if the larvae were not provided with the required diatom mat. The peristomeal growth stage spat measured 288 µm X 218 µm in diameter. On the 16th day of post-fertilization, the shell became almost flat like the adult and shell colouration started as violet and but still transparent. At this stage the animal measured 820 µm X 670 µm in diameter.

The process of metamorphosis was completed and the larva transformed into the juvenile when the first respiratory pore formed at the anterior end of the shell (Plate XX2). This stage was reached on the 26th day of post-fertilization. The first respiratory pore was formed at a shell length of 2.2 mm. Three respiratory pores were formed when the juvenile reached the shell length of 2.6 mm on the 46th day of post-fertilization. Respiratory pores formed continuously and the previous ones sealed off as growth advanced.

The percentage success of fertilization, hatching, larval rearing and settlement achieved during the present experiment are given in the table 33. By transferring the healthy larvae swimming on the top two thirds of water and discarding the rest avoided the introduction of contaminants. Approximately one half of the initial mortality consisted of veligers that failed to settle and metamorphose. More mortality occurred during the first week of post-settlement. Antibiotic treatment of the larval rearing containers resulted the reduction in mortality of larvae during rearing period. The

antibiotics, oxytetracyclin and penicillin, were found to be effective in reducing the mortality of the larvae as well as the settled spats.

As specific inducers are necessary for the settlement success, coralline algal extract obtained by crushing the coralline red algae, were added to some of the settling containers. The containers, which received algal extract, had more number of settlements (about 70%). In the containers with only diatom mat, the settlement percentage was around 30%.

6. DIATOM CULTURE

Maintaining an adequate film of good diatoms for growth in the juvenile rearing tanks is a major concern in the abalone culture process. In the present study, it took 3 to 4 days to get a uniform thin layer of diatoms on the walls of the container. Walne's algal culture medium with the same concentration used for culturing *Chaetoceros* spp. was added to the culture container and gave good results. The water from the container with diatom mat could be used to inoculate another container. When the diatom growing containers were exposed to bright sun light, over-growth of green filamentous algae on the walls of the container was observed.

7. JUVENILE REARING

The settled spats were transferred to the juvenile rearing container after 15 days of post settlement. Juveniles were actively feeding on the retained healthy diatom mat on the walls of the container. Supplementary feeding with fresh seaweeds gave good results. The juveniles, which were fed with *Ulva lactuca* had green and white colouration on their shells (Plate XXI2). Whereas, those that fed on the coralline red algae attached on the rocks had reddish brown colour on their shells (Plate XXI1).

These were the evidences for dietary pigmentation in the juveniles of *H. varia*. 15 to 20 numbers of juveniles were kept in one container during the experimental juvenile rearing period. Water exchange was done only twice in a week. The animals, which were fed with red coralline algae, had a shell length of 11.32 mm after 200 days of post fertilization. While, the shell length for the juveniles fed with green filamentous algae and *Ulva lactuca* were 9.70 mm and 8.96 mm respectively. The average growth of juveniles fed with three different diets during different periods of larval rearing is shown in the tables 28 to 30 and figures 32 to 35. The average daily growth of juveniles fed with the three different diets has also been calculated and is shown in the table 27 and fig. 36. The juveniles fed with green filamentous algae showed retarded growth.

The survival percentage of juveniles fed with coralline red algae was 77% after 200 days. It was 66% for the animals fed with green filamentous algae and 80% for the animals fed with *Ulva lactuca* (Table 31).

PLATE XVII

LIFE CYCLE OF *HALIOTIS VARIA*

1. Fertilized egg
2. Two cell stage
3. Four cell stage
4. Morula
5. Trochophore larva inside
6. Early veliger larva
7. Late veliger larva
8. Gliding larva
9. Settled spat
10. Metamorphosed spat
11. Adult abalone

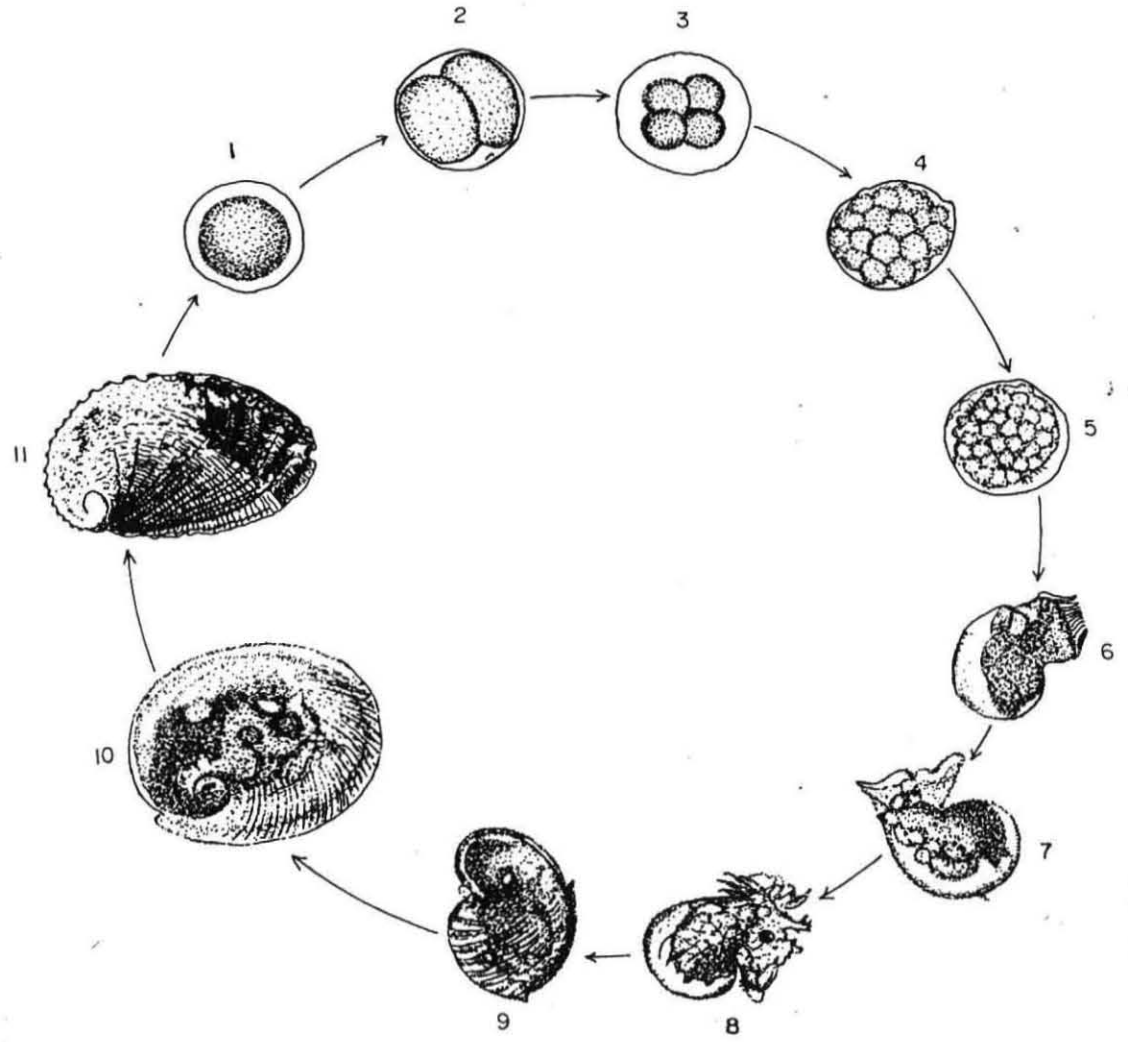
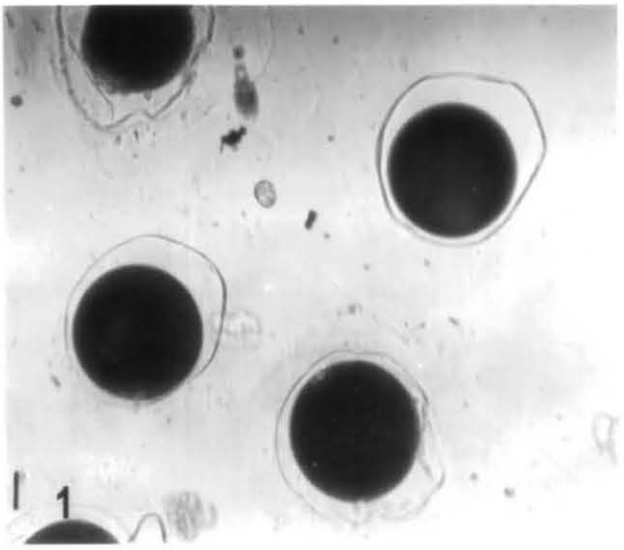


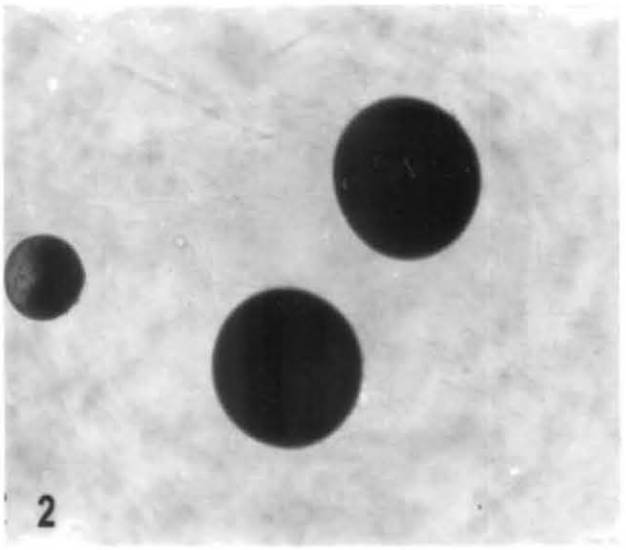
PLATE XVIII

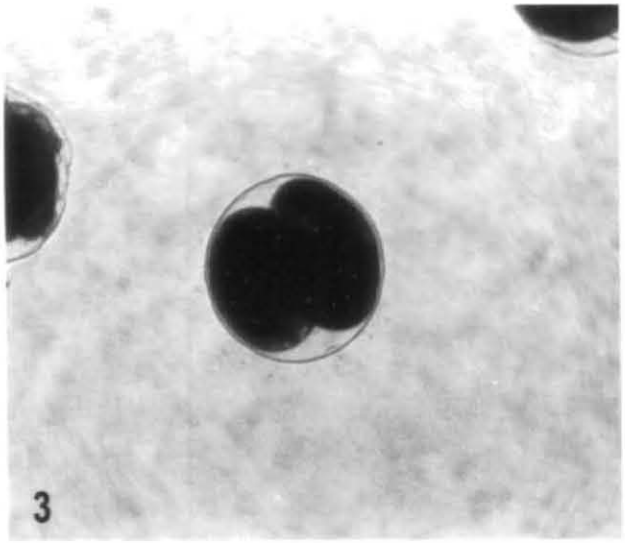
Developmental stages of *Haliotis varia*

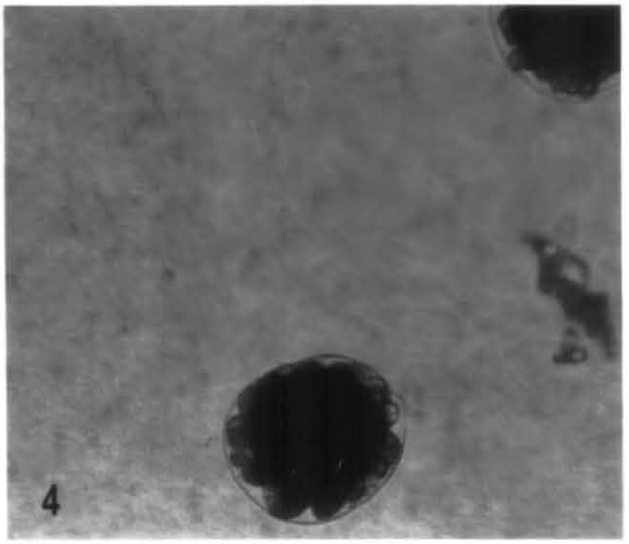
1. Fertilized eggs
2. Spawned immature eggs
3. Two cell stage of the developing egg
4. Morula stage
5. Trochophore larvae rotating inside the egg membrane
6. Hatching out trochophore larva

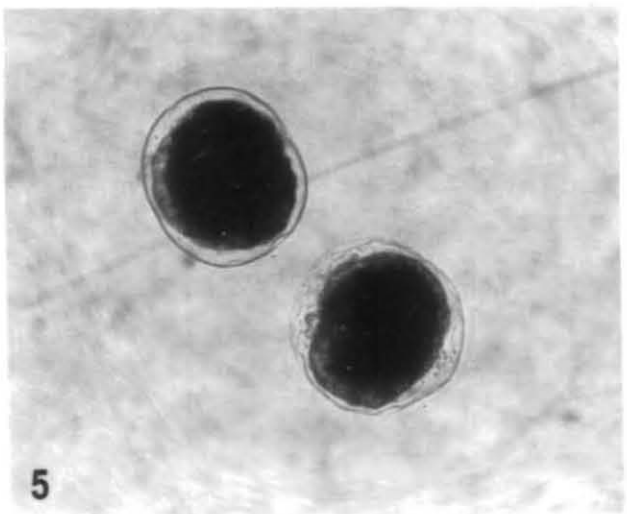
PLATE XVIII











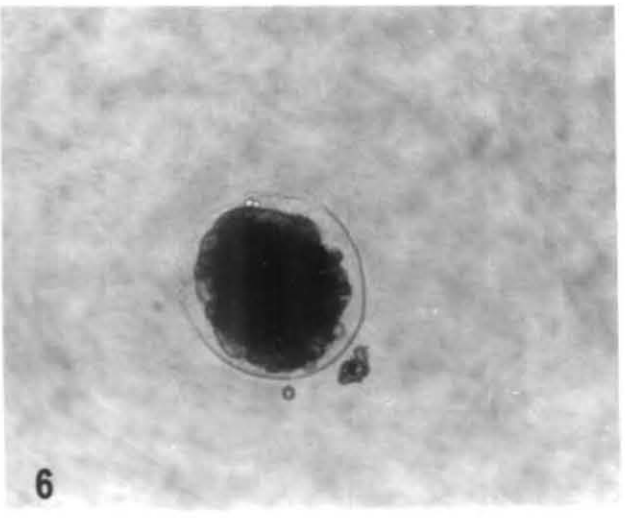
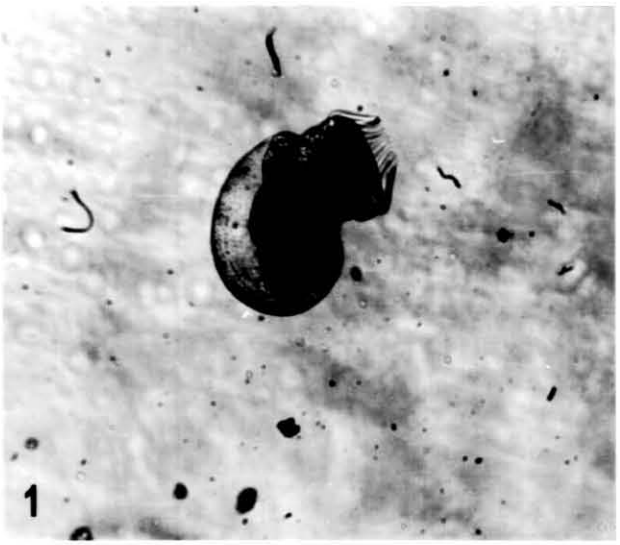
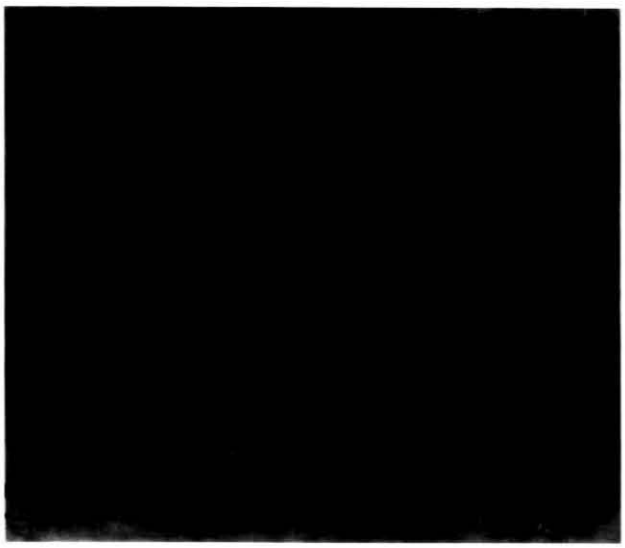


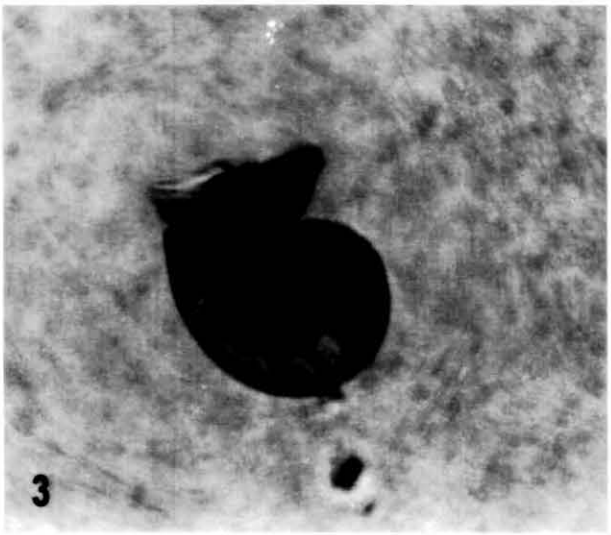
PLATE XIX

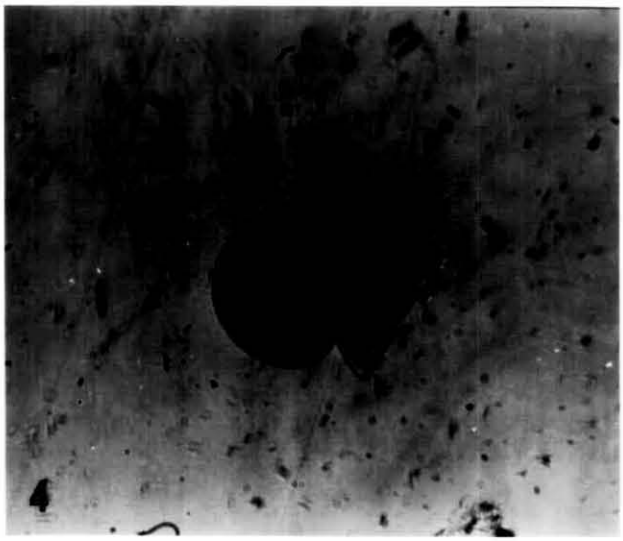
1. Early veliger larva
2. Late veliger larva with retracted velum
3. Late veliger larva with extended velum
4. Gliding larva with eye spots
5. Settled spat of *H. varia*
6. Peristomeal growth stage of *H. varia*

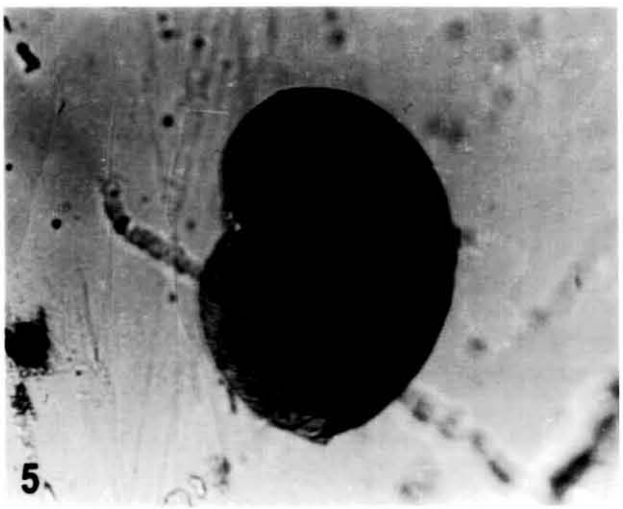
PLATE XIX











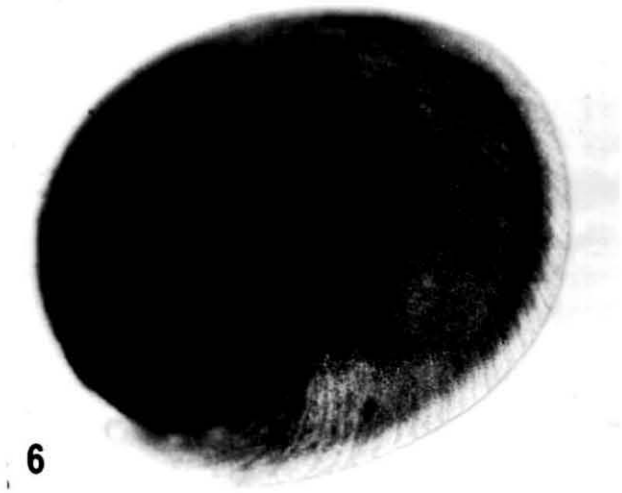
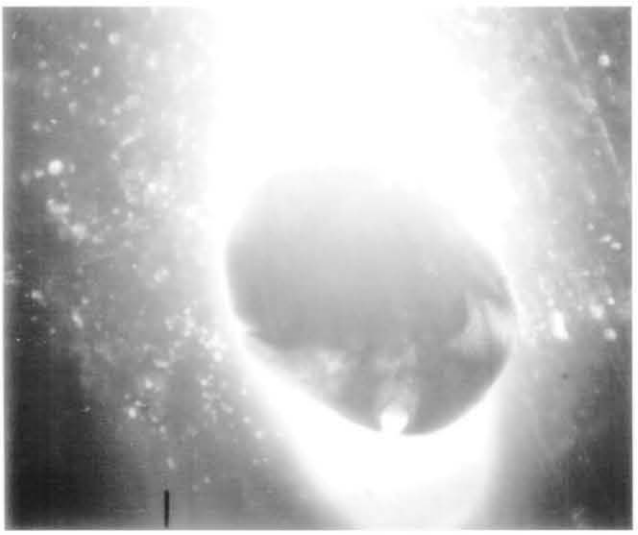
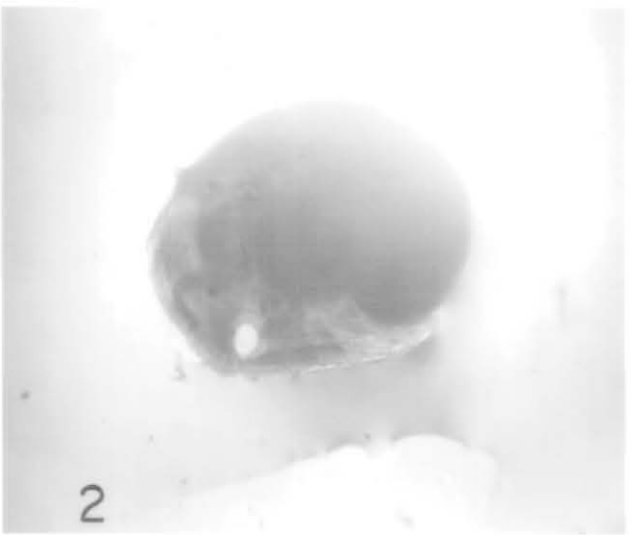


PLATE XX

1. Juvenile *H. varia* started the formation of first respiratory pore
2. Juvenile *H. varia* with fully formed first respiratory pore
3. Juveniles of *H. varia* attached to the mat of coralline red algae

PLATE XX





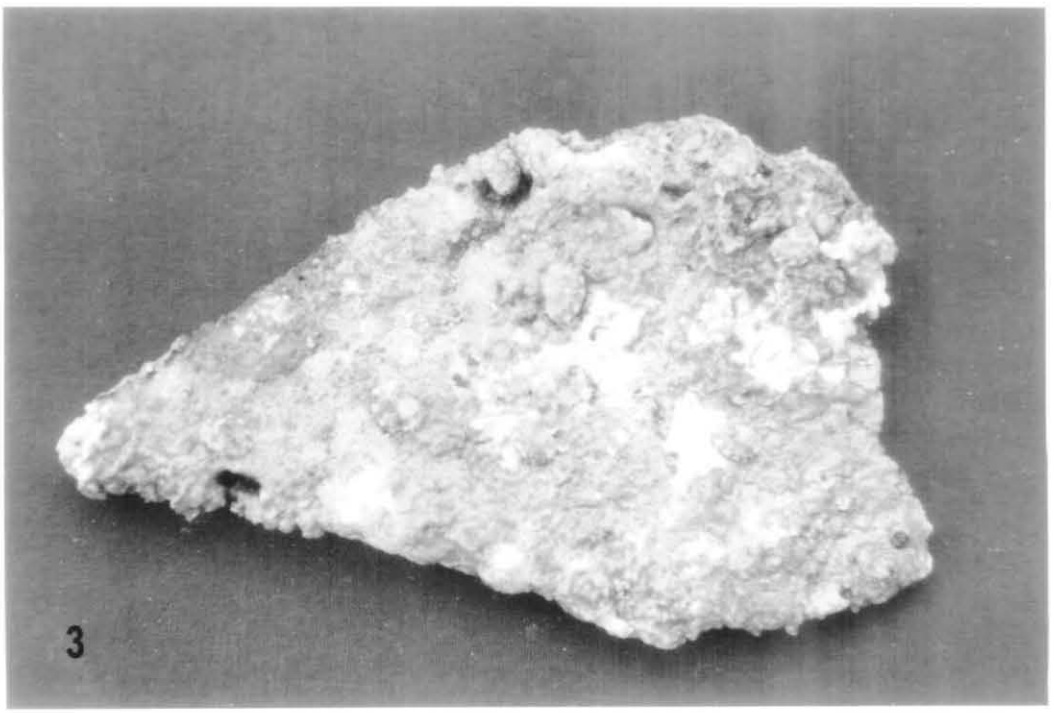
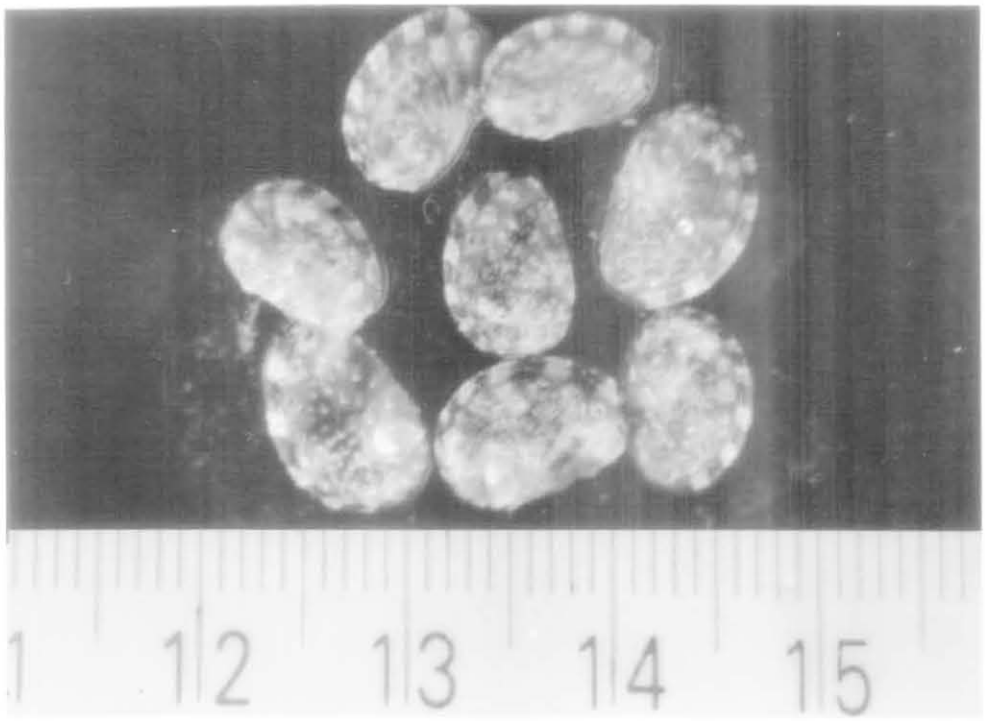
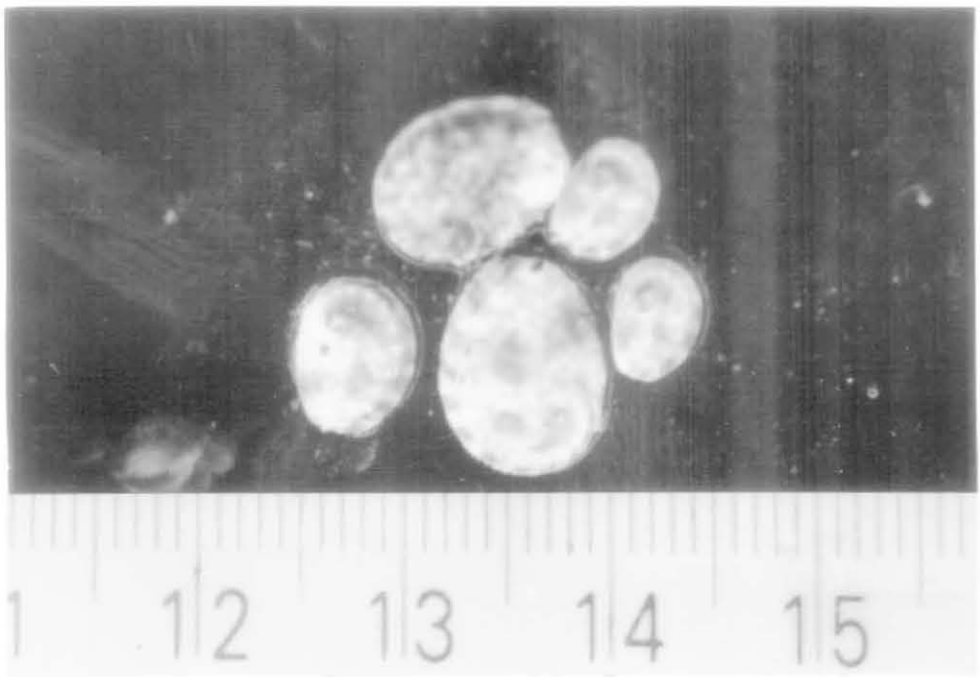


PLATE XXI



1.Juvenle *Haliotis varia* fed with coralline red algae



2. Juvenile *Haliotis varia* fed with seaweed *Ulva lactuca*

Table 26. Average growth of larvae of *H. varia* during rearing period

Days of culture	Length in mm
1	0.180
2	0.210
4	0.230
6	0.288
16	0.820
22	1.000
33	1.350

Table 27. Average daily growth of juveniles of *H. varia* fed with different diets

Period (days)	Average daily growth (ADG) Shell length (μm)		
	Coralline red algae	Green filamentous algae	*Ulva lactuca*
71 – 105	80.853	29.147	88.000
106 –138	92.219	60.063	33.000
139 – 170	33.226	51.563	21.968
171 – 200	37.655	58.379	25.069

Table 28. Average growth of juvenile *H. varia* fed with coralline red algae

Days of culture	Shell length (mm)	Shell width (mm)
70	3.500 ± 0.919	2.185 ± 0.741
105	6.249 ± 0.856	4.569 ± 0.695
138	9.200 ± 1.214	6.616 ± 0.712
170	10.230 ± 0.865	6.920 ± 0.624
200	11.322 ± 0.911	8.108 ± 0.523

Table 29. Average growth of juvenile *H. varia* fed with green filamentous algae

Days of culture	Shell length (mm)	Shell width (mm)
70	3.500 ± 0.919	2.185 ± 0.741
105	4.491 ± 0.902	3.418 ± 0.502
138	6.413 ± 1.011	4.950 ± 1.211
170	8.010 ± 1.021	6.044 ± 0.995
200	9.703 ± 0.812	7.210 ± 0.985

Table 30. Average growth of juvenile *H. varia* fed with seaweed *Ulva lactuca*

Days of culture	Shell length (mm)	Shell width (mm)
70	3.500 ± 0.919	2.185 ± 0.741
105	6.492 ± 0.541	5.088 ± 0.569
138	7.548 ± 0.365	5.860 ± 0.854
170	8.229 ± 0.882	6.253 ± 1.112
200	8.956 ± 1.105	6.672 ± 1.132

Table 31. Percentage survival obtained for juvenile *H. varia* fed with different diets

Days of culture	Survival percentage		
	Coral red algae	Green filamentous algae	*Ulva lactuca*
71	100	100	100
105	89	77	100
138	77	66	100
170	75	64	85
200	75	60	80

Table 32. The optimum level of hydrographic parameters for the larvae of *H. varia* during the rearing period

Parameter	Optimum range
Temperature (°C)	24.0 – 27.0
Salinity (ppt)	33.0 – 36.0
pH	8.1 – 8.6
Dissolved Oxygen (mg/l)	4.25 – 5.64

Table 33. Percentage success of fertilization, Hatching and Settlement during the larval rearing period of *H. varia*

Process	Percentage success
Fertilization	40 – 60
Hatching	70
Settlement	70 - 80

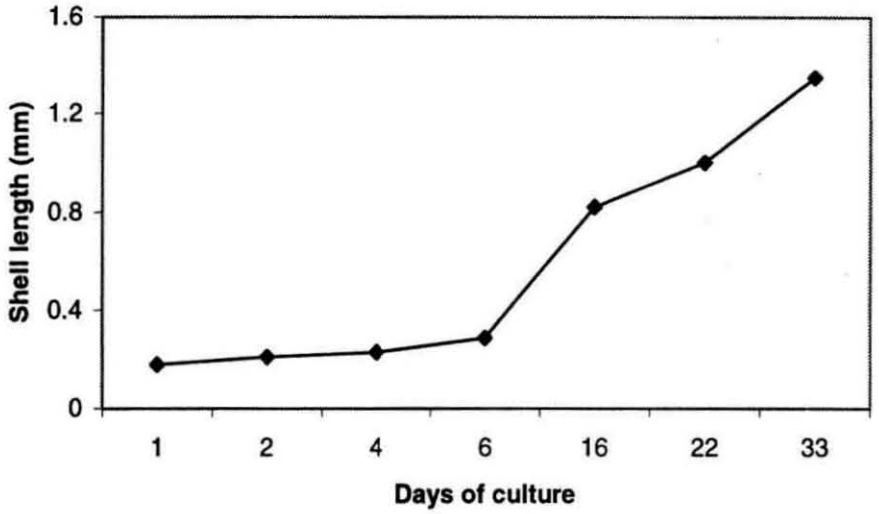


Fig. 31. Growth of the larvae of *H. varia* during the larval rearing period.

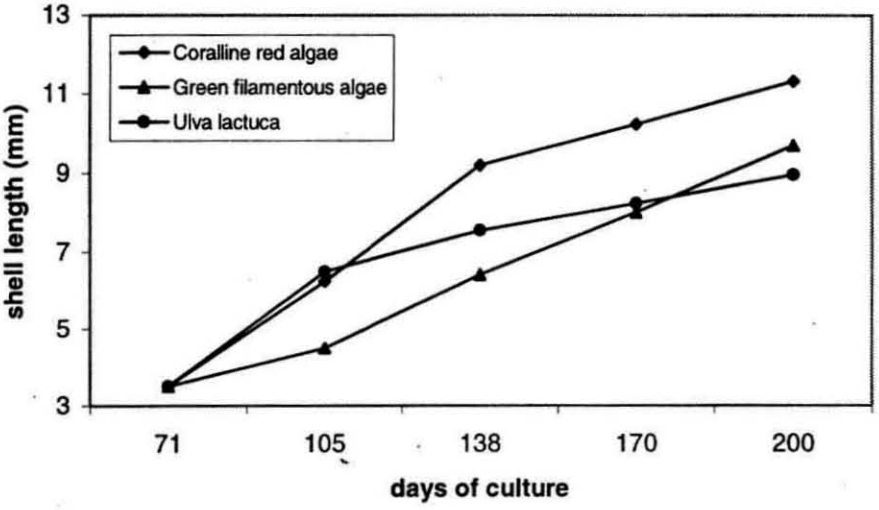


Fig. 32. Growth of the juveniles of *H. varia* fed with three different diets.

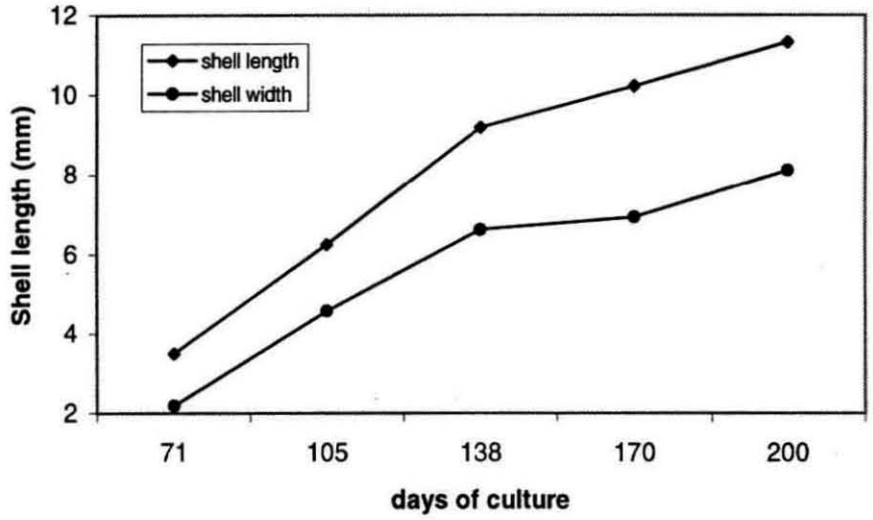


Fig. 33. Growth of the juveniles of *H. varia* fed with coralline red algae.

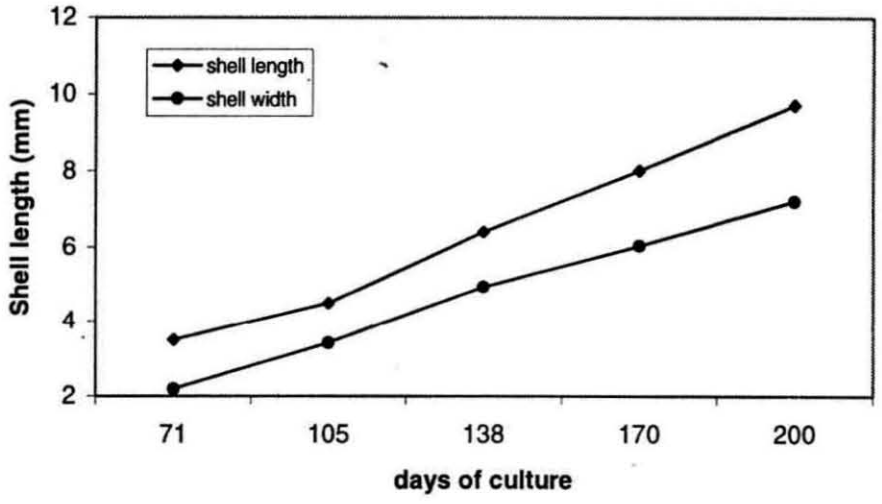


Fig. 34. Growth of the juveniles of *H. varia* fed with green filamentous algae.

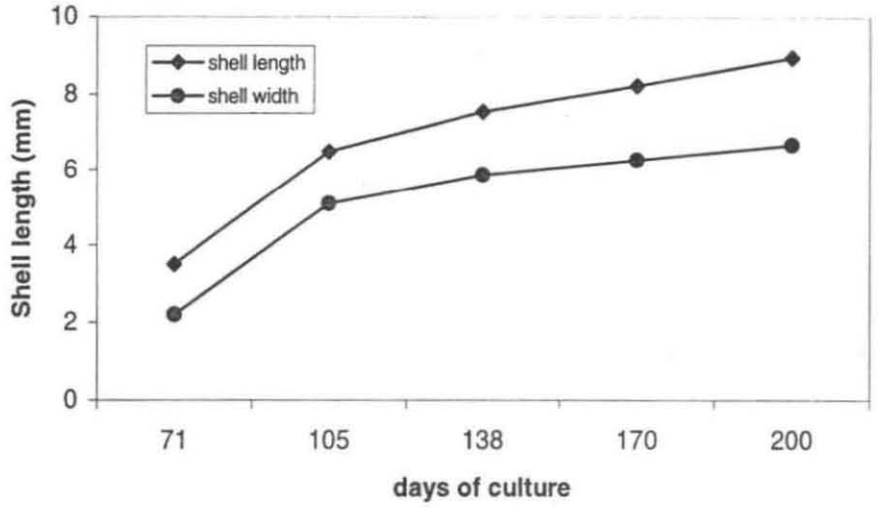


Fig. 35. Growth of the juveniles of *H. varia* fed with sea weed *Ulva lactuca*

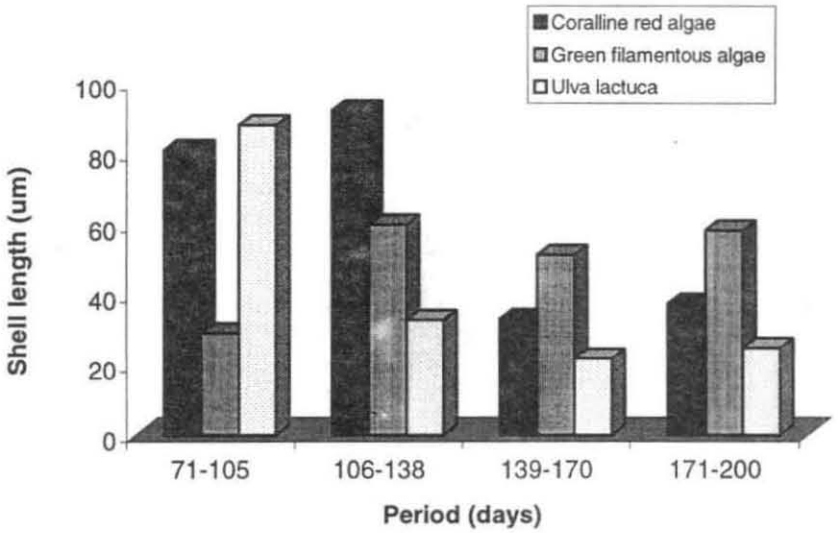


Fig. 36. Average daily growth of the juveniles of *H. varia* fed with three different diets.

DISCUSSION

The breeding season of *Haliotis varia* at Tuticorin extends only from December to March. Ripe male and female abalones could be obtained on one or two day/s before new moon and full moon days. When the collection was made after full moon or new moon days, most of the abalones were in spent stage indicating mass spawning of abalones during this period in the natural habitat. Such lunar periodicity was reported in the top shell, *Trochus niloliticus* by Hahn (1989a), in which the brood stock spontaneously spawned in the evening of new moon day. They exhibited a lunar spawning cycle super imposed on a nocturnal foraging cycle

The high survival during transportation in evening or early morning hours was mainly due to low temperature. The mortalities observed after transferring the abalones to the brood stock maintenance tank were due to the injuries inflicted to the foot during collection. These observations indicate the significance of collection methods of the abalones from the rock substratum. When the water exchange was done frequently in the brood stock maintenance tank, some abalones spawned in the tank itself. The results in the present study indicate that the stress caused by the duration and mode of transport is adequate to bring on spawning by *H. varia*. Genade *et al.* (1988) noted similar observations in *H. midae*.

In the present study, only abalones, which were mature in the natural habitat, were used for spawning experiments. Induced maturation has not been tried in *H. varia* so far. To ensure the availability of ripe animals for longer periods, methods for out of season conditioning should be investigated for *H. varia*. These methods could include *inter alia* saturation feeding (Morse *et al.*, 1978), temperature control and adequate feeding (Uki and Kikuchi, 1984). Artificial conditioning has been

successfully reported in other abalone species, like *H. discus hannai* (Kikuchi and Uki, 1974a), *H. gigantea* (Kikuchi and Uki, 1975), *H. fulgens* (Leighton *et al.*, 1981), *H. discus* (Kafuku and Ikenoue, 1983), *H. rufescens* (Ebert and Houk, 1984), and in *H. corrugata* (Hooker and Morse, 1985).

Presently nothing is known about the nutritional requirements of *H. varia*. *Ulva lactuca* and *Polysiphonia* spp. were given as the feed for abalone brood stock. Though both the seaweeds were consumed by the abalone, no reliable results were obtained for concluding the *ad libitum* feed for *H. varia*. Feeding of *Ulva lactuca* and *Polysiphonia* spp. by the animal was confirmed by the presence of both the seaweeds in the abalone gut by analyzing the gut contents.

Desiccation method of spawning inducement was found to be successful during the present experiment. It is concluded that this method is adequate for *H. varia* with more advanced gonads, which are approaching the natural spawning period. The abalones exposed to air for 2 hours gave good results. When animals were exposed to air immediately after the transportation, high mortalities were noted due to stress. In many abalone culture systems, induced spawning by hydrogen peroxide (Morse *et al.* 1977, 1979a; Morse, 1984; Hooker and Morse, 1985) and UV irradiation (Kikuchi and Uki, 1974b; Uki and Kikuchi, 1982, 1984; Ebert and Houk, 1984; Jarayabandh and Paphavasit, 1996) were the routine methods of spawning inducement. Ebert and Houk (1984) consider UV irradiation inducement of spawning, one of the significant contributions to abalone aquaculture.

In *H. varia*, successful spawning was obtained during the evening or night hours, when the temperature was lowest. Similar observation was made in *H. asinina* by Capinpin and Hosoya (1995), where abalone spawned from about 10 pm to 3 am.

In most of the spawning and fertilisation experiments conducted in abalone, the male and female spawners have been kept separately and fertilisation was accomplished by mixing the eggs and milt immediately after spawning (Kikuchi and Uki, 1974c). But in the present experiment, the male and female abalones were allowed to spawn in the same container, which allowed fertilisation to occur at the same time. Successful fertilisation was obtained in *H. asinina* with similar technique by Capinpin and Hosoya (1995) The disadvantage of this method is the loss of control over the fertilisation process (Hahn, 1989b). So the best option is separating ripe male and female abalones and fertilising the eggs artificially. But further basic research is needed, particularly on the factors that control maturation and optimal sperm density and gamete viability.

In the present study, spawning inducement in *H. varia* was tried by adding hydrogen peroxide to water as described by Morse *et al.* (1978). Because, the abalones used for these trials were not fully ripe, the result was unsatisfactory. Only males responded to hydrogen peroxide treatment. The technique of induced spawning of gravid *H. varia* using hydrogen peroxide needs standardization to obtain successful results.

No reliable data were obtained on the fertilisation rate in *H. varia*. By rough estimation it was found to be around 40-60% in the present experiment. The fertilisation rate of freshly spawned red abalone (*H. rufescens*) gametes reported was 65% at 21°C (Ebert and Hamilton, 1983). In *H. varia*, eggs were fertilised within one hour of spawning. As *H. varia* is smaller than the temperate abalone species, the fertilised egg diameter (180-200 μm) was also smaller than that in the temperate species. Diameter of the fertilised egg reported is 230 μm in *H. discus*, 280 μm in

H. sieboldii, 270 µm in *H. gigantea* (Ino, 1952), 230 µm in *H. iris* (Harrison and Grant, 1971), and 200 µm in *H. ruber* and *H. sorensini* (Leighton, 1972). The diameter of the fertilized ova was 212 to 222 µm in *H. midae* (Genade *et al.*, 1988).

Two of the most critical procedures in cultivating abalone larvae noticed in the present study were the collection of ova from the spawning container and the transfer of veliger stage larvae to the larval rearing container. These procedures are critical because, they present possible contamination sources. Bottom of the spawning container contained fecal matters of adults and the water column contained excess milt in suspension. So it is important to avoid the transfer of these contaminants to the hatching container along with the fertilized ova. Ebert and Houk (1984) reported that these were the major critical procedures in the cultivation of the red abalone, *H. rufescens*. When the eggs were kept in the spawning container for more than four hours after spawning, that adversely affected the hatching rate.

It is important that the fertilized eggs are transferred to the hatching container. During transfer, if the excess sperms were not removed properly the hatching rate decreased. The development of the fertilized egg observed for *H. varia*, is more or less within the time confines of other abalone species like, *H. rufescens* (Ebert and Houk, 1984), *H. diversicolor supertexta* (Liu *et al.*, 1987) and *H. midae* (Genade, *et al.*, 1988).

Scrupulous sanitary procedures should be applied to maintain a clean larval rearing container. The bottom water of the hatching container, which contains shed egg membranes, undeveloped or aberrantly developed ova can be avoided by transferring only 'high rising' veliger larvae from upper two third of the water column to the larval rearing container.

The trochophore and early veliger larvae of *H. varia* are phototactic as described in other haliotids by Ino (1952), Koike (1978), Ebert and Houk (1984), Pena (1986), Liu *et al.* (1987) and Genade *et al.* (1988). The yellow green pigmentation of the egg yolk is retained by the trochophores and the veliger larvae. Leighton (1972) and Genade *et al.* (1988) have also recorded this phenomenon for other species. In *H. varia* the rearing period ranged only from 4 to 5 days at 27 °C. Inverse relationship between larval rearing period and water temperature has been reported in many of the abalone species (Ebert and Houk, 1984; Pena, 1986). The larvae of *H. varia* are lecithotrophic as other abalone larvae. However, Jaeckle and Manahan (1989b) reported that the trochophore and veliger larvae of *H. rufescens*, previously considered to be non-feeding, were not energetically independent of their environment, because they could take up dissolved free amino acids from seawater.

One third of the initial mortality experienced during the present study was in the larval rearing period. The mortality could be reduced by adding antibiotics and it was found to be effective in controlling larval mortality. Morse *et al.* (1979b) have demonstrated the significant role of bacterial growth at the onset of late larval and early post larval mortality. They have achieved temporary prophylaxis by the addition of high concentrations of specific antibiotics to the culture system. Attack by ciliates also was a major problem in the larval rearing container, which could be eliminated by frequent water exchange with clean filtered seawater.

One of the critical stages in the life history of marine invertebrate larvae occurs during the termination of the planktonic or dispersive stage (Slattery, 1992). The transformation from larva to juvenile involves two distinct processes: settlement and metamorphosis (Crisp, 1974; Chia, 1978; Hadfield, 1984). Larval settlement was

achieved within the seventh day of post-fertilisation in *H. varia* at 27 °C. A mat of benthic diatoms comprising mainly of *Navicula* spp. and *Nitzschia* spp. was used as the substrate for larval settlement. Larval settlement of *H. coccinea canariensis* at 15 °C took place 8 days after fertilisation (Pena, 1975). The attachment behaviour of the larvae of *H. varia* was observed approximately on the fourth day post-fertilisation. In *H. diversicolor* it was observed at approximately 54 hours of post-fertilisation (Bryan and Qian, 1998). The red abalone, *H. rufescens* exhibited settlement behaviour at 15 °C only within 6 to 7 days of post-fertilisation (Ebert and Houk, 1984).

Competent larvae of *H. varia* swim downwards and side wards to the walls of the container. They periodically attach their foot to the substratum and crawl. Larvae detached from the substratum and began to swim if they were not attracted to the attachment site. Similar behaviour has been reported for *H. discus hannai* (Seki and Kan-no, 1981a), *H. diversicolor* (Bryan and Qian, 1998) as well as other marine benthic invertebrates (Crisp, 1974; Burke, 1984). This behaviour contrasts to that described for *H. rufescens*, which is reported to commit metamorphosis by exploring a substratum (Morse *et al.*, 1979a; Morse, 1990).

In the present experiments creeping larvae of *H. varia* successfully settled and metamorphosed on the mat of diatoms containing *Nitzschia* spp. and *Navicula* spp.. Similarly, diatom films induced attachment and metamorphosis in *H. discus hannai* (Seki and Kan-no, 1981) and *H. rufescens* (Slattery, 1992). Bryan and Qian (1998) reported that veliger larvae of *H. diversicolor* were stimulated to attach on diatom films and may also be stimulated by certain species of bacteria within these films. The chemical origin of the inductive cue is unclear. Bacteria associated with the diatoms or with the foot of the abalone may be primarily responsible for the inducement of

attachment (Bryan and Qian, 1998). Bacterial films induce attachment in other species of mollusc (Fitt *et al.*, 1990) and may affect abalone larvae as well.

In the present study, the attachment of abalone larvae on the vertical sides coated with diatom films indicates that these larvae are not merely narcotized by components from these sources as primarily suggested (Akashige *et al.*, 1981; Pawlik, 1990). Similar conclusions were made by Bryan and Qian (1998) in *H. diversicolor.*

In the natural environment, abalone larvae settle exclusively on crustose coralline algae (Saito, 1981; Morse and Morse, 1984; Shepherd and Turner, 1985; McShane *et al.*, 1988). Similarly the spats of *H. varia* were seen in the rocks with crustose red algae in the natural habitat. Morse *et al.*, (1979a, 1980) discovered that γ-aminobutyric acid (GABA) – mimetic peptide and phycoerythrobilin, both sequestered at the surface of crustose red algae, were settlement-inducing agents. During the present experiment in *H. varia,* crustose red algal extracts prepared by crushing the scraped algae from the coral stones were added to some of the settling containers. The containers with algal extracts gave better settlement than those with only diatom film. However, detailed investigation is needed to reveal the influence of coralline algal extracts on the settlement and metamorphosis in *H. varia.*

Kawamura and Takami (1995) noted that extracellular mucous from the benthic diatom species, *Navicula ramosissina* and suggested it as an important source of food materials from immediately after metamorphosis to 800 μm shell length. It is important to provide continuous supply of fresh diatom films for the metamorphosed abalones. As the grazing by the juveniles is vigorous the diatom mat is cleaned up within short periods. It is undesirable to allow a thick, filamentous diatom film to develop on the walls of the container because this obstructs the young abalone's mobility and harbors

micro predators. In the present experiment it was increasingly difficult to maintain adequate and continuous diatom films. Alternate exposure of the juvenile rearing container to sunlight gave good results for maintaining the diatom growth.

Another critical step in the seed production of *H. varia* was the thinning of the settled spats by transferring them to another container with fresh diatom coating. Damage was experienced to the delicate shells of the settled spat during thinning because no anesthetics were used during transfer as described by Prince and Ford (1985). Transferring the spat using a paintbrush was found to be effective for *H. varia*.

Growth of the juveniles was faster in elevated temperatures. New shell is added mostly to the anterior of the shell with less growth on sides and posterior. In the present experiment, fully formed first respiratory pore was observed within 27th day of post-fertilisation at 27 °C. It was 8 weeks in *H. rufescens* (Ebert and Houk, 1984). Growth rate of juveniles sharply increases after the first respiratory pore is formed. The juveniles fed with crustose red algae had faster growth than those fed with both the green filamentous algae and *Ulva lactuca*. The major food, the juveniles of *H. varia* come in contact in the natural habitat, is the coralline algae attached to the coral stones. So in natural bed the coralline algae are the major food source for the juveniles of *H. varia*. The other two algae also can be used as the supplementary food for the juveniles of *H. varia*.

The results in the present experiment show that shell colour is affected by the food consumed by the juvenile. The juveniles fed with *Ulva lactuca* had formed greenish white shells, while those fed with coralline red algae, had formed reddish brown shells. Similar dietary shell pigmentation is reported in *H. midae* by Genade

et al. (1988). This property can be utilised to produce quality abalone pearls by altering the irridiscence of the shell interior.

Major advantage in the seed production of abalone is the non-requirement of supplementary feeding during larval rearing period unlike many bivalve species. The larval rearing period of *H. varia* extends only to 4-6 days, which reduces the possible risks associated with larval rearing. Findings of the present study are important, as this would give insight to produce abalones using naturally available diatom species for settlement and metamorphosis istead of expensive chemicals like GABA. The application of various strategies for induced maturation, spawing and settlement in *H. varia* would require further research to bring these process under complete control●

Chapter V

SUMMARY

SUMMARY

1. The specimens of *Haliotis varia* used in the present study were collected from intertidal areas at Tuticorin and Mandapam of Gulf of Mannar. The investigation of the cytological aspects of reproductive biology was carried out using standard histological and electronmicroscopical methods.

2. The gonad of *H. varia* is a conical colourful organ and comprises a part of the conical appendage, which is formed by gonad, digestive gland and part of the stomach. It can be viewed externally, pulling the mantle by a needle. The gonad, as evident from light microscopic studies, consists of a large lumen, which is bounded by a germinal epithelium with a base of parallely running strands of connective tissue. The outer gonad wall is similar in both the sexes and consists of an epidermis with a thin layer of cuticle. Parallely running strands of trabeculae act as supports to the gonad with one end attaching the outer gonadal wall and the other end attaching to the walls of the digestive gland. Cuboidal primary oogonia, which are produced by the germinal epithelium, can be seen near the inner wall of the ovary. The ripe ovary is exclusively filled with vitellogenic oocytes. In the testis, the spermatogonia are seen arranged near the inner walls of the testis and around the connective tissue tubules.

3. Based on the morphological changes such as in colour and shape of the gonad, percentage of gonad covering the conical appendage, gonadosomatic

index and histological changes during the course of gonad maturation, six maturity stages are identified which are early maturing, late maturing, ripe, partially spawned, spent and an indeterminate stage. The characteristics of each stage are described in detail.

4. The gonadosomatic index calculated for each maturity stage of male and female shows an increase as the gonad maturity advances and the highest values recorded were in the ripe stages. The hepatosomatic index was higher in the maturing stages. A negative correlation is observed between the GSI and HSI values indicating the utilization of nutrients from the digestive gland during gonad growth.

5. The size at first sexual maturity of *H. varia* at Tuticorin is at a shell length range of 18-20 mm for males and 22-24 mm for females. In the Mandapam population size range for first sexual maturity among males recorded is 20-22 mm and that for females is 22-24 mm. So there are population differences as well as sexwise differences in attainment of sexual maturity in *H. varia*.

6. The sex ratio in both the stations recorded during the study period is not significantly different from 1:1 ratio.

7. The fecundity of *H. varia* at Tuticorin is found to range between 15,160 in the animal with a shell length of 26.66 mm and 2,75,663 at a shell length of 48.04 mm. A curvilinear relationship was obtained between fecundity and shell length and linear relationship between total body weight and between fecundity and ovary weight.

8. The oocyte size frequency profiles indicate that the ripe ovary is filled exclusively with vitellogenic oocytes. There are no oocytes discernible in the spent stage ovary. This indicates that the spawning in *H. varia* is for a short period. There is a short resting period observed and immediately after that gametogenesis starts.

9. The electronmicroscopic studies of the male and female gonads revealed that a number of distinct developmental stages are involved in the gametogenesis of *H. varia*. The developmental stages of male gametes are spermatogonia, spermatocytes, spermatids and mature spermatozoa. The spermatogonia, which are produced by the germinal epithelium of the testis, measure 5.33 μm in diameter. The primary spermatocytes are smaller than spermatogonial cells and the chromatin is dispersed in the oval nucleus. The proacrosomal vesicles, which are the precursors of the acrosomal vesicle of the mature spermatozoa, make its appearance in the primary spermatocytes. The mature spermatozoan of *H. varia* consists of an anterior conical acrosome and barrel shaped nucleus (together constitute the head piece), a mid piece and flagellum. The nucleus is intensely electron dense. The midpiece is formed by four rounded mitochondria, which are grouped around the proximal and distal centrioles. The flagellum is slender and long. The spermatozoa without flagellum measure approximately 2.8 μm in length.

10. As in other molluscs the oogenesis in *H. varia* involves two distinct processes; proliferative and differentiative. A number of nuclear as well as cytoplasmic changes is observed during the progress of oogenesis. The major changes include the formation of the germinal vesicle, synthesis of RNA in the germinal

vesicle and storage in the cytoplasm and formation of variable numbers of yolk granules (vitellogenesis). Based on the changes in size as well as cytoplasmic contents of the oocyte, five stages of oocytes such as oogonia, previtellogenic oocytes, late vitellogenic oocytes, mature oocytes and degenerating oocytes are distinguished in *H. varia*. The ultrastructural details of these stages are described in detail.

11. The cytoplasm of mature oocyte is filled with both protein as well as lipid yolk. In *H. varia*, vitellognesis is chiefly autosynthetic. The presence of various membrane specializations comprising rough and smooth endoplasmic reticulum in close proximity of yolk granules confirms the autosynthetic nature of vitellogenesis. However, there is also a chance for heterosynthetic production of yolk materials. Small round cortical granules are observed in the periphery of the cytoplasm of mature oocyte. Microvilli are seen embedded in the vitelline membrane of mature oocyte. Degeneration of the oocytes is observed not only in mature oocytes but also in early vitellogenic oocytes.

12. Based on the percentage occurrence of different maturity stages of gonad and monthly changes in the gonadosomatic index, the annual reproductive cycle of *H. varia* at Tuticorin and Mandapam stations were studied for a period of 15 months. Almost all the stages of gametogenic activity were observed throughout the year of sampling. The results indicate that *H. varia* of Tuticorin and Mandapam stations follow a more or less definite pattern of annual reproductive cycle with a short breeding period extending from December to March in Tuticorin and November to February in Mandapam. The spawning

activity commences from December and continues to March and the animals enter into an active recovery phase thereafter.

13. Among the hydrographic parameters studied during the study period, the temperature values fluctuated much over the period and seasonal low values were found to coincide with the breeding season of *H. varia*, both at Tuticorin and Mandapam stations. Similarly, salinity values were lower during the breeding season. But no significant correlation was obtained in the pH and dissolved oxygen values with gonadosomatic index in the entire study period.

14. Estimation of various biochemical parameters such as protein, carbohydrate, lipid and ash in the foot, digestive gland and gonad of male and female *H. varia* during different maturity stages revealed that there is a depletion of some of these body resources from the somatic tissues during maturation. The depletion is found to be mainly due to the translocation of these substances to the gonad for the synthetic activities during gametogenesis and partly for meeting the energy requirements of the animal during the final stages of maturity. The ovary lipid and protein contents showed an increase in the gonad as maturation advances. The lipid content in the ripe ovary is much higher than that in the ripe testis, indicating the role of lipids in the process of vitellogenesis. The fluctuation in the parameters such as ash and moisture in the various tissues were irregular during the course of gonad maturation. The carbohydrate contents in the foot tissues showed a slight increase immediately after spawning and it is concluded that it might be due to the vigorous feeding by the animal after spawning.

15. The seed production of *H. varia*, which was achieved under controlled conditions in the laboratory are described in detail and documented with photographs of various developmental stages. The different larval stages of *H. varia* are described in detail. During the experiment, the veliger larvae were successfully induced to settle and metamorphose on the mat of diatoms comprised of *Nitzschia* spp. and *Navicula* spp. The hatched out larvae are lecithotropic till they reach settlement. Settlement is observed 4-5 days of post fertilisation. First respiratory pore is formed on the anterior side of the juvenile on 27th day of post-fertilisation at 27 °C. It is found during the present study that obtaining and maintaining adequate diatom mat during the settlement and metamorphosis phase of the larvae is a major concern in the seed production of *H. varia*.

16. Juveniles of *H. varia* were cultured for a period of 200 days by feeding with three different types of feeds such as coralline red algae, green filamentous algae and seaweed *Ulva lactuca*. Of these, those fed with coralline red algae showed the best and consistent growth. The shell colour of the juveniles was found to be affected by the feed taken. The juveniles fed with *Ulva lactuca* had formed greenish white shells while those fed with coralline red algae had formed reddish brown shells.

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